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THE EFFECT OF SEVERE HEMORRHAGE, SEASONAL TEMPERATURE AND DIURNAL VARIATION ON BLOOD LACTATE IN THE DOG^{1, 2}

MAJOR M. SWAN

From the Department of Physiology, the University of Kansas

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The blood lactate response in the dog following an acute, severe hemorrhage exhibits a quick rise, followed by a slow decline, and reaches by the end of the first twenty-four hour period after the hemorrhage a level at, or below, the pre-hemorrhage value. This work was first established by Riegel (1927).

Yamada (1937) subjected mature, healthy rabbits to carefully controlled temperature for varying periods of time. When the temperature was 40°C. or 50°C., he noted a considerable increase in blood lactate. Although the animals remained in the high temperatures from thirty minutes to one hour, it required some three to six hours for the blood lactate to return to the resting level. Truka-Tuzson (1940) made ten to twenty determinations of blood lactate per month on different persons throughout a nine-month period from September through June. His lactate values as expressed by the monthly mean varied directly with the monthly mean temperature. Dill *et al.* (1940) found an increase in concentration of blood lactate of approximately 50 per cent when determined in Benoit, Mississippi, as compared to the values found in the same human subjects in Boston, Massachusetts.

An attempt has been made to ascertain if there is a diurnal variation in the blood lactate in the normal, resting dog. In addition, a study has been made of the variation from dog to dog and of the possibility of using some central figure as a norm in referring to the expected blood lactate concentration. In table 1 are given some representative values of blood lactate taken from the literature and other sources. It is interesting to note the extreme variation in values, not only from author to author but also from the low to the high values given by any particular author. The percentage variation, in all but one case, is at least 100 per cent.

¹ This work submitted as partial fulfilment of the requirements for the degree of Doctor of Philosophy in Physiology at the University of Kansas.

² A preliminary report of part of this work was presented before the American Physiological Society in 1941; This Journal **133**: P466, 1941.

Such variations are not the only ones which ought to be considered when evaluating the data dealing with blood lactate in relation to physiological and experimental manipulations. The literature contains little reference to any extended investigation of the blood lactate concentration with respect to the variation which might be expected in an unanesthetized, resting subject from time to time, and the regularity of such variation, *i.e.*, diurnal variation.

The present investigation attempts to answer the following questions: (1) Is there a secondary, delayed hyperlactacidemia following severe, acute hemorrhage? (2) What time relationships are exhibited by the blood lactate response? (3) Is the blood lactate of healthy dogs affected by seasonal changes in tem-

TABLE 1
Typical normal blood lactate values from various authors

AUTHOR	NUMBER OF SAMPLES	VALUES		
		Low	High	Average
Man				
		<i>mgm. per cent</i>	<i>mgm. per cent</i>	<i>mgm. per cent</i>
Clausen (1922).....	3	21.8	32.1	28.2
Barr, Himwich and Green (1923).....	6	14.0	25.2	19.2
Long (1924).....		10.0	20.0	
Schultze (1926).....		9.0	13.0	11.0
Dog				
Gaglio (1886).....		17.0	157.0	
Collazo and Lewicki (1925).....	3	19.6	134.0	58.5
Houget (1933).....	13	10.0	69.0	32.0
Binet and Klukowski (1933).....	66			28.0
Edwards, Brouha and Johnson (1938).....		5.4	14.6	
Ivy and Crandall (Personal communication).....	135	6.0	25.0	12.9
Swan (Present study).....	234	2.0	13.3	10.8

perature? (4) Is there a diurnal variation in the blood lactate in resting, healthy dogs?

To determine the effect of severe hemorrhage on the blood lactate three male and three female dogs were kept in individual cages throughout the experiment. Feeding and watering were done at scheduled times. All samples of blood were withdrawn at the same hour on each sampling day. Complete resting conditions were maintained for each animal. Two additional animals—one male and one female—were used as controls.

To establish basal blood levels on the six experimental animals, samples were taken between 7 p.m. and 9 p.m. on at least two successive days before the hemorrhage. These samples were 6 cc. amounts.

Hemorrhage was achieved by cardiac puncture while the dog was held by an assistant. From 25 to 32 per cent of the total calculated blood volume was

removed in each case. No samples were taken after the hemorrhage until at the expiration of the first twenty-four hours for five days. Thereafter, samples were taken every forty-eight hours through the ninth day.

In work on the effect of seasonal temperatures on blood lactate, two dogs—a female, no. 9, weighing 10.2 kgm., and a male, no. 10, weighing 24 kgm.—were confined in a small, unheated but fully enclosed house constructed of light wooden sheeting. This structure offered complete shelter from wind, rain and snow but afforded little protection to temperature changes. The inside temperature followed within 1° to 3°C . the outdoor temperature.

The thermometer used was the Bristol Recording Thermometer.³ Continuous temperatures throughout the twenty-four hour period were thus available. This instrument provided readings which served as the semi-official temperatures for the community.

Uniform feeding and watering procedures were used for the dogs. The food was a balanced, commercial product which was given to them once each day at 5 p.m. The dogs' conditions and weights improved during the experiment, indicating a healthy state at all times.

Samples were taken from the peripheral leg veins, all four legs being used in rotation. All samples were taken at 11 a.m. every day of the week except Sundays and holidays. Approximately 12 cc. of blood were removed each time a sample was obtained. That such a blood loss was in no way injurious was indicated by the improvement in each dog's condition and vigor.

To insure a state of complete rest in the experimental animal, both dogs were leashed for at least forty-five minutes previous to the time of blood removal. No form of activity was permitted during this rest period.

The experiment was started early in the fall when the temperature daily reached a peak almost as high as during the hottest part of the summer. Samples were taken every other day from each dog for a period of six months. During this period, temperatures approximating those of hottest summer as well as those of coldest winter were registered.

To determine the effect of diurnal variation on blood lactate, dogs were selected from the general dog pen and allowed to rest for forty-five minutes before the first sample was drawn. Food was withheld from the animal during the twenty-four hour period of sampling. Every precaution was taken to insure conditions of complete rest throughout the entire test period.

Males and females were used on alternate test periods. Insofar as possible, a different dog was used for each test period. However, it was found necessary to use one particular dog—a male—for three test periods.

This series of experiments was continued for a twelve-month period; hence ten different dogs were used. Each test period included twenty-four samples, distributed an hour apart throughout the twenty-four hour period. Each twenty-four hour run was made once a month on approximately corresponding days.

METHOD. Samples of venous blood were withdrawn from peripheral veins into glass syringes containing sufficient dry sodium oxalate to prevent coagula-

³ Courtesy of the Kansas Public Service Company, Lawrence, Kansas.

tion. Exactly 5 cc. were measured by an Ostwald pipette and delivered into diluting flasks. To this measured sample of blood were added 8 volumes of N/12 sulfuric acid and 1 volume of 10 per cent sodium tungstate. Glycolysis was avoided by completing the blood withdrawal and precipitation of proteins within five minutes.

The blood was filtered through paper and the carbohydrates removed by the method of Van Slyke (1917). Then after centrifuging, the tubes containing the filtrates were stoppered and stored in the refrigerator. Within a week the lactate content was determined by the method of Friedemann, Cotonio and Shaffer as described by Peters and Van Slyke (1932).

All samples except those obtained in the work on the effect of diurnal variation were run in duplicate, the results agreeing within 2 mgm. per cent in practically

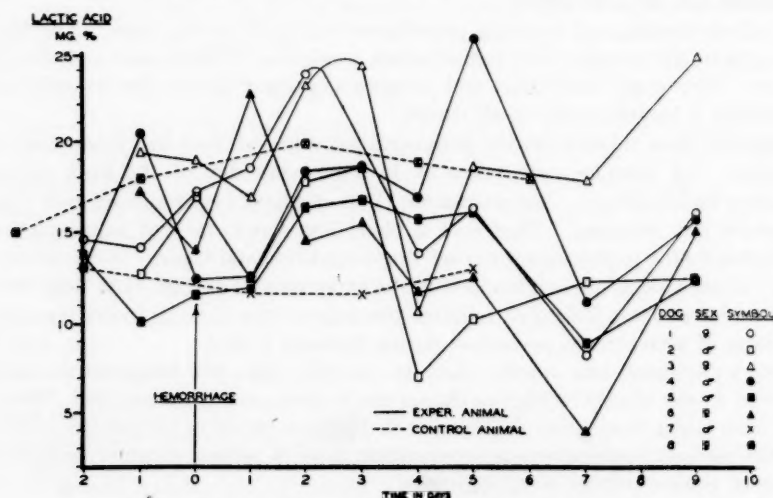


Fig. 1. Effect of severe hemorrhage on blood lactate in dogs

all cases. All blood filtrates were analyzed within a week after removal from the dog and, in the interim, were kept in stoppered tubes in the refrigerator.

RESULTS. *The effect of severe hemorrhage on blood lactate.* In figure 1 a complete record of the data is presented. Examination of the blood lactate values obtained from each dog before hemorrhage indicated that, even in the normal, resting subject, there was considerable variation. This variation was somewhat random in nature. Dogs 1, 2 and 5 showed higher lactate values in the second sample than in the first. On the other hand, dogs 3, 4 and 6 showed a decrease when their second sample was compared to their first.

Forty-eight hours after the hemorrhage, decided increases in blood lactate were found in all dogs except no. 6, a female, whose blood lactate had, by this time, decreased to the basal level. Seventy-two hour samples gave values slightly higher than those of the second day in every case except dog 1, whose sample was lost in the titration.

The blood lactate values obtained on the fourth day were generally lower than any previous results, either normal or after hemorrhage. On the fifth day, values generally higher than on the fourth were found and, in one case, that of dog 4, the highest values for that animal and for the series were obtained.

From this point, samples were drawn every other day. Those obtained seven days after the hemorrhage gave values which, in most cases, were below those previous to the hemorrhage. In all but dogs 2 and 3 the values were the lowest of the whole series. On the ninth day the lactate concentrations had again risen to about the pre-hemorrhage level, although in one dog, no. 3, the concentration was quite high.

While there is considerable variation, yet, in general, it can be noted that the variation before hemorrhage is at random. Twenty-four hours after hemorrhage the lactate levels are, with two exceptions, at the resting level for any given dog.

In five of the six dogs, the lactate concentration was sharply increased at the end of forty-eight hours. Approximately the same high level was present at the end of seventy-two hours. Then, without exception, lower values were found at the end of ninety-six hours. Some of the ninety-six hour values were lower than the pre-hemorrhage levels.

From the ninety-sixth hour to the end of the experiment—where the majority of the levels approached the pre-hemorrhage resting values—the lactate variations occurred in unison. Some variations were of greater magnitude than others, however. Throughout the duration of the recovery period there were periodic fluctuations of considerable extent.

In some way, then, the random behavior of the blood lactate level had been modified by the hemorrhage so that it displayed a periodic nature. It is obviously not totally a response to a deficient oxygen supply to the tissues, even though this may cause a hyperlactacidemia as has been shown by Araki (1891) and many others since. It is difficult to see how the tissues could be adequately supplied with oxygen at the end of twenty-four hours and be inadequately supplied in the forty-eighth hour, although the animal was in a state of rest during both periods.

Doubtless, a hyperlactacidemia during or immediately following hemorrhage, such as Riegel (1927) described, is best accounted for on a basis of oxygen deficiency. Gesell *et al.* (1930) have shown in anesthetized, operated animals that such a hyperlactacidemia is found following a reduction in oxygen content in the respired air. Similarly after hemorrhage, Gesell obtained high blood lactate values. These experiments were of short duration and on animals under conditions not normal. Hence, it may be questionable to attempt a direct transposition of results.

Particularly is this true in view of the experiments of Cook and Hurst (1933). These investigators, using healthy human subjects, found that the lactate content of the blood during rest was the same as during a period of light to moderate exercise. Even when their subjects walked at a rate of three miles per hour no increase in blood lactate could be found in venous blood immediately draining the active muscle groups.

Jervell (1928) in studying the blood lactate concentration in the blood of anemic patients was unable to demonstrate any consistent relationship between hemoglobin percentage or red cell counts and lactic acid. Jervell concluded that deficient oxygenation was seldom, if ever, serious enough in anemic patients to prevent the combustion of the amount of lactic acid normally produced.

Bock, Dill and Edwards (1932) failed to find any significant change in blood lactate of normal men following reduction of inspired oxygen to nine per cent, ingestion of sodium bicarbonate or ammonium chloride or taking moderate exercise. These investigators demonstrated a change in pH and alkaline reserve in some of the above procedures, but they could demonstrate no consistent relationship between hydrogen ion concentration and lactate changes in the blood of their subjects. Summing up their results, in the light of work done by other investigators, these authors remark:

Of the lactic acid present in the blood of a resting subject a small portion may come from muscle activity, the rest presumably from the activity of the central nervous system, various glandular activities, etc. It seems logical to suppose that the ability to reconvert lactic acid to its precursor may vary greatly at the seat of its formation, just as the rate of utilization of oxygen may vary from organ to organ in the body. The problem remains for study along lines differing from those suggested in the past.

It is our opinion, too, that the level of blood lactic acid in the resting subject must be accounted for in a way which differs from any suggested previously. Therefore, we venture such a suggestion.

A possible explanation of the blood lactate response is afforded by linking the lactate level of the subject, whether in ordinary rest or rest following severe hemorrhage, with the showers of leucocytes which are liberated into the blood stream. The evidence, at present, is only suggestive since there is little in the literature concerning extended observations of the leucocyte count as related to other physiological variables. The observations which have been reported, although fragmentary, indicate that there may be some relation between the presence of leucocytes and lactate concentration in the blood of resting subjects.

This is not to be construed as denying the well established fact that blood lactate varies under conditions of physical stress. Our purpose is to call attention to some similar aspects of leucocyte and blood lactate changes.

Drinker, Drinker and Kreutzmann (1918), in one of the few extended experiments on the cellular content of the blood of dogs following large hemorrhage, showed that the discharge of leucocytes into the peripheral blood followed a pattern which exhibits many similarities to the lactate variations in our experiments.

We wish to emphasize that there is no direct relation between our blood lactate and Drinker, Drinker and Kreutzmann's leucocyte values. Such agreement is not to be expected since the two variables were determined on different dogs at widely separated times. The experimental conditions were different, since leucocyte counts were obtained during a study of the effect of repeated hemorrhages and saline infusions under anesthesia, while our lactate values were taken following one severe hemorrhage on normal, resting dogs.

The effect of seasonal temperature on blood lactate. A complete record of the data collected in the experimental period of six months shows considerable variation from one determination to the next, even in the series from the same animal. Even though there is great variation from one sampling day to another, yet this variation is no greater than is the variation observed when several successive samples are taken from a dog during a twenty-four hour period (unpublished work). In addition to the greater variability, dog 9 consistently contained a higher concentration of blood lactate than did dog 10.

There is no relation between the blood lactate changes represented and the day-by-day temperature changes. Neither is there a direct relation between

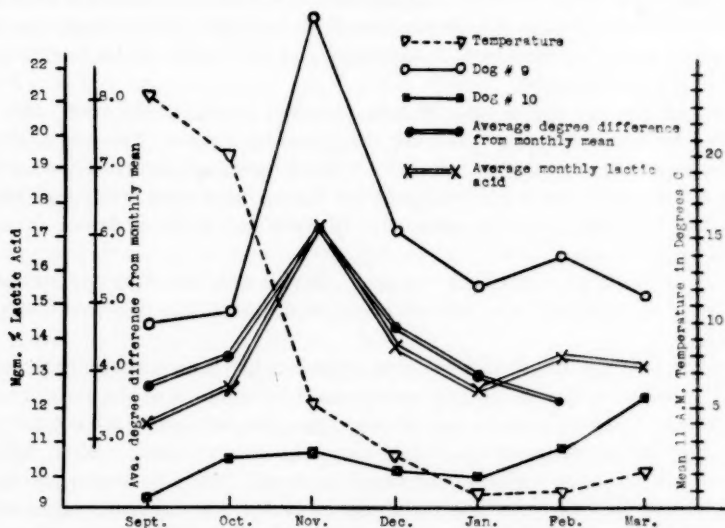


Fig. 2. Average values for blood lactate and temperature. The lactate curve represents monthly mean values. The temperature curve represents the dispersion of the daily 11 a.m. temperature from the monthly mean 11 a.m. temperature.

the average lactate values for a given month and the average 11 a.m. temperature during the month.

However, a general trend in the same direction with respect to both dogs was observed. This similarity indicated that the changes in blood lactate were brought about by the same common agent or agents. But any such blood lactate response may be modified, no doubt, by a multiplicity of factors.

Examination of figure 2 shows a close correlation between the average monthly blood lactate values and a variable which we have chosen to designate the "average degree difference from the monthly mean." This quantity and the statistical mean deviation are identical. It is a measure of the dispersion of the daily 11 a.m. temperature from the mean 11 a.m. temperature for a given month. Surprisingly close agreement is observed between these two variables for the

experimental period. Such behavior is interpreted to mean that results in a blood lactate change but more probably the temperature fluctuates around a central point.

Perhaps, too, a total temperature change may influence the blood lactate, since the February lactate values were higher for both dogs than the mean deviation would seem to warrant. Therefore, it may be that, in addition to the degree of variation as a factor, a large temperature change of a continuous nature was an additional factor. With respect to the March values, it is seen that there is some disagreement here, too. It is felt, however, that too few values are represented upon which to base any definite conclusions.

The effect of diurnal variation on blood lactate. The data indicated that there were two distinct periods of high concentration during a given twenty-four hour test. One high tide occurred about noon and the other about twelve hours later, i.e., near midnight.

The mid-day rise began near 10 a.m., reached a peak at 12 noon, and then subsided to below the mean value for the group by 4 p.m. The mid-night rise was quite pronounced, but it lacked the well sustained character of the mid-day increase. Its peak was a little higher than the mid-day peak, yet it exhibited a somewhat fluctuating nature, since the 12 midnight value is lower than the 11 p.m. or the 1 a.m. values.

One transient rise occurred about 6 p.m. It appeared and disappeared quickly although its magnitude was almost equal to either of the two previously described.

Lactic acid in the blood of the resting organism has been considered the result of acid production by contracting muscles and its subsequent diffusion into the blood stream. Recent experimental work on man failed to account for more than a part of the resting blood lactate as due to such a course. Bock, Dill and Edwards (1932) suggest that resting blood lactic acid "May be a split product of carbohydrate mobilized for the maintenance of the general metabolism of the body." This generalization is so vague that it offers little aid in explaining the facts.

A comparison of the behavior of blood lactate with the behavior of other constituents of the blood under conditions of complete rest reveals the interesting fact that the only constituent in the blood which exhibits diurnal variations of comparable frequency and magnitude is the white blood corpuscle. Further study of the concentration of these structural elements suggests a striking similarity to the concentration of blood lactate.

One careful twenty-four hour study of the diurnal variation of leucocytes was that of Shaw (1927), in which he followed the leucocyte changes in four normal human subjects by performing white cell counts every hour throughout a twenty-four hour period. Sabin *et al.* (1925) studied the normal rhythm of the white cells in resting men covering the daylight hours of 9 a.m. to 4 p.m. only. Tschishikow (1927) studied the so-called digestion leucocytosis in dogs and included a curve showing the rhythm of the leucocytes in resting fasted dogs during the period from 8:20 a.m. to 9:30 p.m. Zirm and Bauermeister (1933) followed

the leucocyte variations on resting hospital patients and obtained results quite similar to those published by Shaw (1927).

These authors obtained evidence of diurnal variations in the leucocyte count. When their values were plotted the resulting curves resemble the curves showing lactic acid changes. In all studies there was a mid-day rise in leucocytes with considerable variation from hour to hour. In fact, Sabin spoke of an "hourly rhythm of leucocytes." In the more extended studies there was also a mid-night swell. The late afternoon rise was present in Shaw's series but came an hour later in Tschishikow's.

The diurnal variations in leucocytes in man along with the blood lactate mean hourly values of our resting dogs are strikingly similar in their time relationships but one must bear in mind that they represent different species.

This marked similarity may be merely an accidental occurrence. Without direct evidence concerning the relationship of the two variables, one would be unjustified in assigning to one the rôle of cause and to the other that of effect, or to both the result of a common cause.

An attractive hypothesis would be to consider the blood lactate in the resting organism, partly at least, the result of the glycolytic activity of the white cells.

It is well known that one of the potentialities of the white cell, particularly the segmented cells, is the production of lactic acid from carbohydrates. Levene and Meyer (1912) first demonstrated leucocytic splitting of dextrose in vitro. Maclean and Weir (1915) demonstrated that both erythrocytes and leucocytes glycolyzed dextrose in vitro but that the activity ratio of leucocytes to erythrocytes, cell for cell, varied roughly from 200:1 up to 1000:1 in favor of the leucocytes. They concluded that, in normal blood, the white cells probably exert greater glycolytic effect than the red cells even though they are present in much smaller numbers. Falcon-Lesses (1927) proved that leucemic blood in vitro displayed greater glycolytic activity than normal blood.

DISCUSSION. *The effect of severe hemorrhage on blood lactate.* The significant point to be observed concerning the relationship of the delayed hyperlactacidemia and leucocyte increase following severe hemorrhage in dogs is that the direction and rate of variation of the two are similar. They simply show that, when an animal is subjected to a severe blood loss, the blood picture with respect to either variable can vary drastically from day to day in the recovery period. At this point, all that can be said is that future work may show a relationship between the lactate content of the blood and the leucocyte count.

The effect of temperature on blood lactate. It may be that the mechanism operating to bring about an increase in blood lactate in the dog when the daily temperature fluctuates around the mean point is of a nervous nature, involving the structures usually acting in temperature regulation. Yamada (1940) has shown that, in rabbits, after bilateral splanchnicotomy there is not obtained the usual hyperlactacidemia following temperature changes. This might indicate a possible involvement of the adrenal medulla, the secretion of which has been shown to influence the blood lactate level (Cori, 1925).

The effect of diurnal variation on blood lactate. Considering, then, the above

demonstrated facts regarding the rhythmic variations of leucocytes in the blood stream and also the blood lactate changes, one may be justified in adopting as a hypothesis the conception that lactate changes in the blood of the resting dog are, in a measure, dependent upon and/or associated with the variations in numbers of leucocytes. This hypothesis must be regarded as provisional until further work of a direct character determines whether or not it can be regarded as valid.

SUMMARY

1. An average value for blood lactate in the resting dog of 10.8 mgm. per cent was found. A total of 234 samples was used.
2. The blood lactate level during recovery from severe, acute hemorrhage of 25 to 32 per cent of the total blood volume in three male and three female dogs was studied by taking daily samples of peripheral blood. Each daily sample was drawn at the same hour, the subjects being in complete rest.
3. After hemorrhage high blood lactate values were found in five dogs at the forty-eighth hour and were still present at the seventy-second hour. One dog varied from the general response in that the blood lactate peak was observed on the twenty-fourth hour.
4. From the third day until the end of the ninth day, when the study ended, the blood lactate curve showed well defined peaks and troughs, indicating a rhythmic variation as compared to the random blood lactate variation of the normal resting subject.
5. No significant difference according to sex was observed.
6. Evidence was given which suggested that the variation in leucocytes may account for some of the phenomena following severe hemorrhage.
7. Blood lactate in dogs did not seem to vary with the day by day fluctuation of the 11 a.m. temperatures.
8. No direct relation was shown between monthly mean blood lactate and monthly mean temperature.
9. A close correlation did exist between monthly mean blood lactate and "temperature mean deviation." By "temperature mean deviation" is meant the dispersion of the daily temperature from the monthly mean temperature.
10. It is suggested that a nervous mechanism may be responsible for the regulation of these variations, since other work has shown, in the rabbit at least, that bilateral splanchnicotomy can check lactic acid production following large temperature changes.
11. There was a well defined diurnal variation in the blood lactate of the resting, normal dog.
12. The twenty-four hour period exhibited two crests. One at mid-day lasted well into the afternoon. The other occurred approximately at mid-night and was of shorter duration than the one at mid-day. It may be, however, of greater magnitude than the one at noon.
13. A third crest was observed in the late afternoon near 6 to 7 p.m. This peak was shorter, more spiked in appearance, and more inconstant in occurrence.

14. Data from the literature describing the diurnal variation of leucocytes are presented showing a similarity between the changes in concentration of leucocytes and blood lactate during the twenty-four hour period.

15. A provisional hypothesis linking the two phenomena is suggested.

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MUCUS, ACID AND WATER SECRETION IN THE STOMACH FOLLOWING THE INJECTION OF PILOCARPINE^{1, 2}

FRANKLIN HOLLANDER AND JULIUS STEIN

From the Laboratories of The Mount Sinai Hospital, New York City

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It is generally believed that pilocarpine, injected subcutaneously, "activates chiefly the production of gastric mucus and enzymes", whereas the secretion of HCl and water are stimulated only secondarily and to a small extent. A critical review of the literature on the gastro-secretory activity of pilocarpine (5), however, reveals that this conclusion is supported by only a fraction of all the evidence reported on this subject since 1875, when jaborandi was first introduced into Europe. Many investigators³ obtained considerable outputs of acid secretion, in some cases comparable with the response to food or to histamine injection. Also, in many instances the content of visible mucin in pilocarpine juice was found to be but little greater than in histamine or post-prandial secretion. The following investigation was undertaken in an effort to resolve the conflict among these many reports, by the use of a different experimental approach to the problem. The question of pepsin secretion was not considered in the present study.

EXPERIMENTAL DETAILS. For the various experiments here described we employed 6 dogs, provided with greater curvature pouches of varying degrees of vagal innervation, i.e., 3 Pavlov, 2 Heidenhain, and 1 of our own type of vagal pouch (8). The mouth of each pouch was provided with a "sphincter", thus making it possible to collect the secretion by either of the following methods: 1, the usual, *continuous collection technique*; 2, the *discontinuous collection or retention technique*, whereby the gastric juice is allowed to accumulate within the small stomach in the absence of the collecting catheter (6). The latter investigation showed that even a small rubber catheter, when held in the pouch throughout an experiment, will induce the secretion of an appreciable amount of visible mucus and consequently some reduction of acidity, though less than is likely to be obtained with a permanent cannula. With the retention technique, however, this mechanical stimulation of mucus is reduced to a minimum (its duration is never greater than half a minute) although the expression of mucus by rubbing of mucosa against mucosa, as a result of increased muscular activity, may occur in the retention experiments as well as in the continuous collection ones.

No experiment was started unless the pouch had been inactive for at least 30 minutes before injection of the stimulus, as indicated by the complete absence of

¹ A preliminary report of this work was presented before the American Society of Biological Chemists in 1938 (9).

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³ Bibliographic references of historical interest alone are furnished in detail in the aforementioned review and for this reason they are kept to a minimum in the present, experimental report.

free acid. Pilocarpine hydrochloride (hereafter represented by P) solutions, prepared fresh each time, were injected subcutaneously in doses of 1.0, 0.5, 0.05, 0.025 or 0.01 mgm. per kgm. body weight; the first two of these will sometimes be referred to collectively as the upper dosage range, the other three as the lower. Histamine hydrochloride (hereafter represented by H) was administered similarly in a dose of 0.3 mgm. per kgm., which represents about the same number of moles as does the 0.5 mgm. dosage of Pilocarpine HCl. Body weights of the dogs varied from 4.5 to 14 kgm. Records were kept of the frequency and intensity of vomiting and also of intra-pouch bleeding as evidenced by the color of each specimen; an index system of 0, 1, 2 or 3 pluses was found adequate for the comparative purposes of this aspect of the study. Duration of an experiment (total time) was measured from the time of injection (irrespective of the latent period of secretion) until the return of the secretory rate to its basal level. The observations also included the total volume of secretion collected every 15 minutes and the volume of visible mucin which sedimented in the graduated centrifuge tube after being spun for 10 minutes at 2700 R.P.M. Acidities were determined by semi-micro titration and total chlorides by a micro-modification of the Volhard method (10). Neutral chloride was calculated by difference between total chloride and acidity. It should be noted that the phenol red end-point for total acid is not as reliable in the presence of mucin as in its absence, but this is not true for the brom-phenol blue end-point for free acidity. Dry weights, both for dissolved organic solids and for ash, were determined by methods previously described (4).

As a measure of the response of the parietal cells to different procedures or agents, several criteria were available to us: 1, the maximum free and total acidities attained in any single experiment (expressed in milli-normal concentration); 2, the total quantity of acid (calculated as milli-equivalents of HCl) secreted throughout the experiment; and 3, the average acidity throughout the experiment. In order not to prejudice our evaluation of secretory responses, we employed all 3 of these criteria. For the third, a simple arithmetical mean of the titration values for all the specimens appears to possess less validity than a weighted mean which takes cognizance of variations in volume of secretion as well; i.e.,

$$\text{average acidity} = \frac{\sum (\text{acidity} \times \text{volume, for each specimen})}{\sum (\text{volume for each specimen})}$$

Since it is sometimes stated that P stimulates the secretion chiefly of the organic constituents of gastric juice whereas H stimulates HCl and water, we included the total volume of fluid as another measure of secretory activity, and also determined the percentage of soluble organic and inorganic solids in a number of selected specimens. In order to simplify the comparison of data obtained under different experimental conditions, the results are reported in terms of an average for all the experiments in each group performed under essentially the same conditions, rather than in terms of the individual experiments. This statistical procedure was considered valid because the secretory responses to gastric stimu-

lation gave no indication of being characteristic of the individual dogs, whether they possessed a vagotomized pouch of the Heidenhain variety or one of the 2 types of vagal pouch.

RESULTS. *General observations.* The responses to the various doses of P, when only the continuous collection technique was employed, are summarized in table 1; those based on the retention technique are presented in table 4. The

TABLE 1
Mucus, acid, and fluid responses to pilocarpine and histamine; continuous collection experiments

(Data reported as averages for entire groups of comparable experiments)

	STIMULUS					
	Pilocarpine					Hista- mine
	Dosage (mgm./kgm.)					
	1.0	0.5	0.05	0.025	0.01	
1. Number of experiments per group.....	9	9	6	4	4	9
2. Total volume of precipitated mucin per experiment (ml.).....	1.22	1.06	0.25	0.20	0.20	0.09
3. Total duration of secretion per experiment (hours)...	4.00	2.81	1.25	1.19	1.06	1.83
4. Rate of mucin secretion (ml. per quarter hour).....	0.081	0.099	0.053	0.056	0.046	0.014
5. Total quantity of acid (meq. $\times 10^{-3}$).....	2.01	1.77	0.17	0.09	0.03	2.57
Free/Total.....	2.25	1.93	0.19	0.10	0.04	2.63
6. Maximum acidity (mN)....	111	119	66	49	18	143
Free/Total.....	119	126	73	54	25	147
7. Mean acidity (mN).....	84	102	59	44	15	131
Free/Total.....	94	112	65	50	22	135
8. Total volume of fluid per experiment (ml.).....	23.4	17.6	2.7	1.8	1.6	19.9
9. Rate of fluid secretion (ml. per quarter hour).....	1.46	1.57	0.54	0.38	0.38	2.72
10. Blood per experiment (crude index).....	16	8	1	0	0	1
11. Number of vomitings per experiment (crude index)....	4.3	1.1	0	0	0	0

frequency of vomiting which occurred with the two highest doses, and the degree of bleeding and of restlessness, were usually so great as to preclude the use of a dose greater than 1.0 mgm. per kgm. with safety to the animal and the experiment. At the lower dosage levels, there was no evidence of bleeding whatever and vomiting was reduced to a negligible amount. Micturition and defecation were induced in many of these experiments and the mucus content of the feces was frequently very high. Salivation was considerably more profuse than gastric

secretion and it started promptly after injection, whereas the latent period for the pouch secretion was sometimes as long as 15 minutes. The gastric fluid was invariably clear, like the secretion obtained in response to H—except for greater amounts of coagulated mucin.

The duration of gastric secretion, following a single injection of P in the upper dosage range, was considerably longer than for the H experiments, in consequence of a relatively long period of gradually falling acidity with the former stimulus. For purposes of comparison a series of experiments with H were also included. As usually happens with this stimulus, its administration was followed by no vomiting whatever and, but rarely, by slight bleeding of mechanical origin.

The output of insoluble mucin by continuous collection. Let us first consider the response of the mucus cells as measured by the volume of visible mucin obtained after centrifuging—the method used by many previous investigators. The standard dose of H gave an average output of only 0.09 ml. per experiment (line 2, table 1), whereas following the injection of P, the volume of precipitated mucin was considerably greater, irrespective of the dose. The quantity increases with dosage of P, that for 1.0 mgm. being 6 times that for 0.01 mgm./kgm. Since the duration of secretion (line 3) also varies considerably with the dosage of P, the mucus output is obviously correlated with the total time (though not linearly), and the response was therefore studied in terms of mean volume per unit time (line 4). This secretory rate likewise shows a downward trend with decreasing dosage, but the ratio between the extreme values is only about 2:1 instead of 6:1. Similarly, the response to 1.0 mgm. of P per kgm. is 14 times the response to 0.3 mgm. of H in terms of mucin volumes, but in terms of secretory rates their difference is only 6-fold. It must be concluded, therefore, that P, at all the dosage levels here employed, yielded more insoluble mucin than did the control experiments with H, whether the situation was studied in terms of absolute quantities or rates of secretion. Compared with the rate of fluid output (line 8), however, the mucin rate values were extremely small being of the order of 2 to 3 per cent of the former in the lower dosage levels and 0.5 per cent in the higher. It might be expected that the absolute amount of mucin would increase with diminished intensity of stimulation, analogous with the responses to electrical stimulation of the vagi reported by others; but in spite of a 100-fold variation in dosage in these experiments the reverse was actually the case.

Acid and fluid secretion by continuous collection. If the total quantity of HCl secreted in any experiment be taken as a measure of the parietal cell response, we find (line 5) that the highest dosage of P gave on the average only 15 to 20 per cent less acid than did H in its standard dosage. Lower doses of P gave correspondingly smaller quantities of HCl, but even one as low as 0.01 mgm. per kgm. yielded an appreciable amount of acid. The data for total volume of fluid (line 8) and rate of fluid secretion (line 9) also reflect this downward trend with dosage, but although the rates never were as high as that for H, the total volume of fluid obtained with 1.0 mgm. of P actually exceeded that with the standard dose of H by 18 per cent—contrary to what might be expected from a large part of the literature. Total duration of an experiment (line 3) also shows a positive corre-

lation with dosage, and the P experiments with the two higher doses lasted very much longer than the H experiments. Analysis of the acid response in terms of the maximum acidity attained in each experiment (line 6), or the average acidity throughout the experiment (line 7), yields a slightly, though not materially, different picture. By both of these criteria, the highest response was obtained at the 0.5 mgm. dosage level, rather than 1.0 mgm., the difference between them being 7 to 8 mN (6 per cent) in terms of maximum acidity and 18 mN (16 per cent) in terms of mean acidities. These differences are of uncertain statistical significance, but if they have any validity at all they can be only a secondary consequence of several other factors operating in combination—like the rates of mucus and fluid secretion and the amount of vomiting. Comparing the response to 0.5 mgm. of P with the corresponding response to 0.3 mgm. of H, we find that both these acidity measures are lower in the P group; in terms of maximum total acidity the difference is 14 to 16 per cent, and in terms of average total acidity it is 17 to 20 per cent. However, these differences, as well as the analogous difference in terms of total quantity of acid, are all sufficiently small so as to indicate that P in these larger doses may still be considered a good stimulus to HCl as well as to fluid secretion, even as compared with histamine. Although the rate of secretion of fluid (line 9) is materially faster in the H experiments than in any of those with P, irrespective of dosage, doses of H somewhat less than 0.3 mgm. per kgm. would undoubtedly have given comparable results even in this respect.

Chloride secretion by continuous collection. Estimations of total and neutral chloride concentrations were performed whenever the specimens were large enough to permit of such determinations parallel with acidity titrations. Total chloride values are generally high with but small variations during any one experiment; values for neutral chloride tend to be correlated inversely with the acidity, as we have previously shown to be the case for H secretion. This relation is indicated by the group averages of table 2, and it is amply demonstrated by analysis of the data for individual experiments. Hence, P is not essentially different from H in respect to chloride concentrations, and the variations which are observed are only such as may be expected from admixture of mucus and parietal cell secretion in different amounts.

Total dissolved solids by continuous collection. The volume of insoluble mucin represents only one aspect of mucus secretion, and crudely at that, since it disregards the mucin which remains in solution. In order to check on this soluble factor, we determined the concentration of dissolved solids in a number of specimens of P secretion; for purposes of comparison, a like number of determinations were made on H juice (table 3). The individual specimens were chosen with a view to their yielding 2 groups of data with approximately the same mean acidities, and also to their being large enough for analysis. In general, volume of specimen (actually, rate of secretion when the time factor is held constant) is correlated positively with the acidity, for P as well as for H and food; in accordance with this the P specimens of table 3 possess a mean acidity considerably higher than any of the corresponding values of table 1 (line 7), and more nearly

equal to the maximum value (line 6) for this same dosage level (0.5 mgm. per kgm.). The 2 groups of data for ash content are in reasonably good agreement with each other, as might be expected from their identical acidities, but the concentration of organic solids in the P secretion is almost twice that in the H secretion. The actual concentration ratio of P to H is 1.8, whereas for precipitated mucin the analogous ratio is about 13 (data from table 1, line 2, adjusted

TABLE 2
Chloride and acidity data; continuous collection experiments

STIMULUS AND DOSE	EXPERIMENT NUMBER	SPECIMENS TITRATED	MEAN Cl CONCENTRATION		MEAN ACIDITY	
			Total	Neutral	Free	Total
			m.N	m.N	m.N	m.N
Pilocarpine 1.0 mgm./kgm.	J-26a	3	156	73	74	82
	J-28	11	159	73	78	86
	J-33	7	157	66	74	85
	J-35	14	147	37	95	107
	J-36	14	160	42	108	116
	J-38	10	155	63	78	85
	J-48	6	151	51	80	95
	J-49	10	152	41	93	109
	J-54	4	157	70	73	79
	Mean	79	155	57	84	94
Pilocarpine 0.5 mgm./kgm.	J-2	7	150	57	68	93
	J-7	3	157	56	90	101
	J-9	7	158	27	120	131
	J-14	7	156	28	120	126
	J-25	12	154	35	104	114
	J-56	7	151	54	84	90
	Mean	43	154	43	98	109
Histamine 0.3 mgm./kgm.	J-12	5	160	14	137	142
	J-13	2	165	28	128	131
	J-24	8	161	13	142	144
	J-30	3	159	20	135	138
	J-32	5	160	38	118	122
	J-34	7	158	28	127	130
	Mean	30	160	24	131	135

for the total volume of fluid per experiment, in line 8). Hence the difference between these two stimuli as regards their output of dissolved organic matter is less than for insoluble mucin, but it is still sufficiently great so that there can be no question of its validity.

Visible mucus by the retention technique. From the foregoing data, it is evident that the P experiments yielded more mucus secretion than did the H experiments,

irrespective of the dosage level of the former, but it does not necessarily follow that the mucus cells were stimulated directly by the pilocarpine, acting at the neuro-glandular junction. From our previous experience with histamine alone and with ingested food we know that some, at least, of this mucus may have been evoked mechanically—in part, by rubbing of the mucosal surface against the catheter, and in part, by being squeezed out of the cells as a result of the increased activity of the gastric musculature. Any pharmacological procedure for controlling these mechanical processes entails the risk of simultaneous influence on the direct stimulation of the mucus cells, if any such exists. The rubbing effect, however, can be eliminated in great measure without this risk by use of the

TABLE 3

Dissolved organic solids and ash of pilocarpine and histamine secretions (filtered); continuous collection experiments

SPECIMEN NUMBER	STIMULUS	ACIDITY		ORGANIC SOLIDS <i>mgm./100 ml.</i>	ASH <i>mgm./100 ml.</i>
		Free <i>mN</i>	Total <i>mN</i>		
23-1	<i>mgm./kgm.</i> Pilocarpine	127	132	0.48	0.06
2	0.5	122	129	0.41	0.08
3	<i>mgm./kgm.</i>	108	115	0.44	0.18
4		123	130	0.28	0.21
5		148	152	0.41	0.29
6		116	123	0.22	0.16
7		137	142	0.32	0.21
Mean		126	132	0.37	0.17
23-8	Histamine	102	106	0.29	0.34
9	0.3	147	150	0.06	0.13
10	<i>mgm./kgm.</i>	99	107	0.44	0.33
11		140	143	0.20	0.14
12		121	126	0.22	0.26
13		143	147	0.14	0.13
14		145	149	0.10	0.12
Mean		128	133	0.21	0.21

retention technique for collecting the secretion. Accordingly we performed 2 series of experiments by this method, using P in doses of 1.0 and 0.5 *mgm.* per *kgm.* respectively. The results are compared in table 4 with corresponding data from the continuous collection experiments.

Although the durations of the 2 groups of retention experiments (line 3) were not significantly different, on the average, from those by the other procedure, the total volume of precipitated mucus per experiment (line 2) and the rate of mucus secretion (line 4) are both distinctly less in the retention group. In terms of total volume of precipitated mucus, the differences are 47 and 58 per cent of the median values for the 1.0 and 0.5 *mgm.* dosages respectively; in terms of secretory rate,

they are 58 and 98 per cent. Because of the crude method of measuring mucus output, and the fact that the frequencies (number of experiments per group) are never greater than 9, these differences are not subject to reliable statistical evaluation. Their validity, however, is confirmed by the data on maximum and mean acidities—both free and total (lines 6 and 7). For both of these criteria and for both the dosages of P, the retention data are invariably greater than the corresponding continuous collection data; the greatest of these differences is 25 per cent of the median, the least is 10 per cent. The differences in terms of mean acidity are greater than in terms of maximum acidity, and since the latter data

TABLE 4

Comparison of secretory responses to pilocarpine with different methods of collecting gastric juice (data reported as averages for entire groups of comparable experiments)

	DOSE OF PILOCARPINE			
	1.0 mgm./kgm.		0.5 mgm./kgm.	
	Method of collection			
	Continuous	Retention	Continuous	Retention
1. Number of experiments per group.....	9	9	9	8
2. Total volume of precipitated mucin per experiment (ml.).....	1.22	0.75	1.06	0.47
3. Total duration of secretion per experiment (hours).....	4.00	4.03	2.81	3.06
4. Rate of mucin secretion (ml. per quarter hour).....	0.081	0.047	0.099	0.034
5. Total quantity of acid (meq. $\times 10^{-3}$).....	2.01	2.94	1.77	1.74
Free/Total.....	2.25	3.36	1.93	1.88
6. Maximum acidity (mN).....	111	125	119	133
Free/Total.....	119	140	126	140
7. Mean acidity (mN).....	84	105	102	122
Free/Total.....	94	120	112	130
8. Total volume of fluid per experiment (ml.)...	23.4	27.5	17.6	15.5
9. Rate of fluid secretion (ml. per quarter hour)...	5.85	6.82	6.26	5.07
10. Blood per experiment (crude index).....	16	15	8	7
11. Number of vomitings per experiment (crude index).....	4.3	3.2	1.1	0.3

are based on fewer than 25 values, they also are not subject to statistical analysis with any degree of confidence. The mean acidities, on the contrary, are calculated for frequencies which vary from 98 to 145 specimens per group of experiments. For such frequencies, the usual significance test for the difference of 2 means is acceptable, and its application indicates that all 4 mean acidity differences are significant at the 5 per cent level of probability. Comparative experiments of this kind were not performed with the lower dosages because of the very small quantities of secretion which these yield and the consequent increase in variability of the observations.

From these findings it may be concluded that, for a given dose of P, the reten-

tion technique yields both higher acidity values and lower mucin values than does the continuous collection procedure,⁴ the data being in accord with the idea that these higher acidities result from diminished secretion of, and neutralization by, mucus. It is interesting that, irrespective of the dosage, the amount of bleeding and the frequency of vomiting appeared to be less in the retention experiments than in the others, even though the quantitative validity of such differences cannot be established. Apart from the influence of these two factors, however, the greater lowering of acidities (from the characteristic value for the parietal fluid, about 167 mN) in the continuous collection experiments must be ascribed to the mechanical stimulation of the mucus cells by the collection catheter. Elimination of this stimulus results in a diminished output (or secretory rate) of mucus and consequent elevation of acidity. Were it possible also to inhibit specifically the motor effects of the P, these differences between the two groups of experiments involving the same dosage of P but different collection techniques would be even greater, i.e., the mucin contents of the retention experiments would be less and the acidities higher than those actually observed.

DISCUSSION. We have found that P, in doses of 0.5 and 1.0 mgm. per kgm., yields concentrations and amounts of HCl, which, though less than those given by 0.3 mgm. of H per kgm., are of the same order of magnitude. No experiment was performed unless there was a complete absence of acid secretion prior to injection of the stimulus, and at no time were 2 stimuli superimposed on each other. Previous investigators failed to take the first of these precautions, and several actually report the simultaneous administration of P with H or food (17). Hence the present study reflects solely the stimulating power of P. As for the secretion of water, the H data are actually intermediate between those for P at the 2 upper dosage levels, but the smaller amounts of both HCl and water, obtained with lower doses of P, in no way affect the conclusion that, *under suitable conditions, P stimulates the flow of gastric HCl and fluid in amounts comparable with H*. The responses from differently innervated pouches were essentially the same, consistent with the observations of others. The identity of the acid/chloride relations for P and H secretion affords further evidence of the essential similarity of these two types of secretion.

Besides the dosage of P, two other factors are responsible for the low concentration of HCl relative to the H results. One is the lower rate of acid secretion, which ordinarily is correlated positively with acid concentration (3, 13), but this was offset by the greater duration of response to a single injection, at higher dosage. As a result the total quantities of acid in the P and H groups were in better agreement than were the corresponding concentration data. The second factor is the greater rate of mucus secretion under the influence of P. Our data (7) indicate that the buffer capacity of this secretion may be even greater than has hitherto been reported; hence though the quantitative response of the parietal cells

⁴ Individual acidities of the continuous collection experiments with P never quite attained the highest values of the H experiments (149/152 mN). The retention experiments, however, actually exceeded these values; with 1.0 mgm. of P the highest individual acidity was 155/160 mN, and with 0.5 mgm. of P it was practically identical 156/160 mN).

may be the same for a pair of P and H experiments, the greater activity of the mucus cells in one of this pair must lower the acidity throughout that experiment. Had we not taken pains to minimize mucosal irritation and trauma, the observed acidities would have been even lower for any set of experimental conditions. These factors, and the several methods of analysis applied to our data on acid output, serve to explain the conflicting results reported by previous investigators of these phenomena.

Concerning mucus output the picture is more involved. P clearly induces greater activity of the mucus cells than does H, as shown by the relative rates of secretion of visible mucin and the data on dissolved organic solids, even for the lower dosages of P. Had the dry weight determinations been performed on specimens of higher acidity, the values for organic solids would have been even lower—because of the negative correlation between the concentrations of dissolved solids and HCl (4)—but the difference between the 2 stimuli would probably have persisted in some measure. Hence P administration undoubtedly increases the output of mucin but there must be considerable doubt about the mechanism of stimulation. In general, P exercises a vagomimetic function in the stomach but histological evidence for the parasympathetic innervation of the mucus cells is lacking. Otherwise one might expect the output of mucus to be considerably greater after P than by "spontaneous" secretion, but this proves not to be so.⁵ Even the volume of mucin relative to that of fluid in the P experiments is small, the ratios being 0.052, 0.060, 0.093, 0.111 and 0.125, for descending dosages.

It has been reported by Uschakow (15) and Vineberg (16) that electrical stimulation of the vagi gives acid secretion if the induced current be strong, but mucus (frequently followed by acid) if it be weak. The data from lines 2 and 4 of table 1 are not at all consistent with this differential reaction because the values decrease with decreasing dosage; also, though the mucin:fluid ratios rise with diminishing dosage, this results from decreasing fluid volumes rather than increasing mucin volumes. On the other hand, the experiments of table 4 demonstrate that a large portion of the mucus output is the result of mechanical stimulation of the mucosa by the catheter. Observations on humans (2, 11) and on gastric fistula dogs (1) have shown that increased motility, even of hunger contractions, increases the "spontaneous" secretion of mucus. Hence, another

⁵ We have compared the rate of mucin secretion for the various groups of P experiments (table 1, line 4) with similar unpublished data on the rate of "spontaneous" secretion of mucin, measured by continuous collection 18 to 24 hours following the last meal. With P, the rates (in ml. per quarter hour) vary from 0.046 to 0.099; "spontaneous" mucus secretion in the absence of free acid (pH 6.0) has a mean rate of 0.10 (20 expts.), and when accompanied by acid secretion (pH 3.0) of 0.03 (17 expts.). The non-acid values represent viscous mucus, whereas the acid values are for insoluble mucin. Had the non-acid "spontaneous" specimens been acidified and centrifuged, their values in terms of precipitated mucin would probably have averaged between 0.05 and 0.07 ml. per quarter hour. If the resting secretion of acid is actually psychic, and therefore vagal, we might expect that the mucus cells would also be stimulated vagally in such experiments and that the acid specimens would contain more mucin than the non-acid ones. The fact that the reverse is true casts further doubt on the parasympathetic innervation of the columnar epithelium.

large fraction of the mucus output after P must be ascribed to such mechanical expression; elimination of the motor component of the response to P without interfering with the secretory component would probably reduce the rate of mucus secretion to an exceedingly low value. H induces no motor activity (12) and therefore only a negligible amount of mucus secretion (6, 14). Whether the output of P mucus can ever be lowered to the level of the H experiments can be established conclusively only by the selective elimination of these secondary mechanisms of stimulation; in the light of the reduction already effected by use of the retention technique of collecting secretion, it seems not at all improbable. Hence it may be concluded that (1) *a major part of the mucus secreted in the P experiments is stimulated mechanically, in several ways, and (2) it is doubtful whether any of this secretory activity is induced by direct action of the drug at the neuro-glandular junction.*

SUMMARY

1. Pilocarpine, when injected subcutaneously into stomach pouch dogs in adequate dosage, stimulated the flow of gastric HCl and fluid in amounts comparable with those evoked by histamine. The secretory-time curves were generally irregular but were characterized by relatively long latent periods and total durations of response to a single injection.

2. Administration of pilocarpine was accompanied also by the secretion of gastric mucus in considerable quantity, especially when the continuous collection technique (with a catheter in situ throughout the experiment) was employed. The rate of secretion, however, did not differ materially from that of the "spontaneous" secretion of mucus in acid-free pouches. Furthermore, a major part of this mucus-secretory activity was eliminated when the retention technique was used for collecting the pouch contents (in the absence of a solid collecting device), thus demonstrating the mechanical (in contradistinction to direct pharmacological) origin of the mucus which is ordinarily evoked by this stimulus. Evidence from other laboratories indicates also that a considerable part of the mucus is squeezed out from the surface epithelial cells by the muscular activity induced by the pilocarpine, even in the absence of rubbing by a foreign body. Hence it is questionable whether any of the mucus-secretory activity is induced by direct action of the pilocarpine at the neuro-glandular junction.

3. In agreement with previous observations, high dosages of the drug (0.5-1.0 mgm. per kgm.) induced restlessness, salivation, mucosal bleeding, vomiting and defecation. Oozing of blood in the pouch occurred to a greater extent in the continuous collection experiments than in those performed by the retention technique.

4. This investigation gives further evidence of the importance of the retention technique for certain kinds of quantitative investigations of gastric secretion.

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A COMPARISON OF THE CONFIGURATION IN THE ELECTROCARDIOGRAM OF ENDOCARDIAL AND EPICARDIAL EXTRASYSTOLES^{1,2}

H. E. HOFF AND L. H. NAHUM

From the Department of Physiology, McGill University, Montreal, and the Laboratory of Physiology, Yale University School of Medicine, New Haven

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Lewis considered the theory of "limited potential differences" as an explanation of the electrocardiogram to be established by the observation (1) that the initial deflection of extrasystoles elicited from the endocardium of the right ventricle in the dog was downward, while that from the overlying epicardial surface was upward. This observation was explained by assuming that in the first instance a limited region of potential difference was created with the negative poles oriented toward the inside of the heart, while in the second instance the dipoles were reversed because the primary direction of propagation was from without inward.

The results of experiments on external stimulation of the right and left ventricles, summarized in other papers (2, 3), suggest that there is another interpretation of the phenomenon described by Lewis. Extrasystoles elicited from the right ventricle do show downward initial deflections in certain leads when the surface is stimulated at almost any point except in an area around its center. (By the center of a ventricle is meant that region of its surface at its lateral border which is equidistant from all points along the septum.) These downward deflections have already been shown to have their origin in excitation of the left ventricle (2).

Conversely, it has been shown that extrasystoles elicited from the left ventricle show downward initial deflections in all leads only when the point of stimulation is in a restricted area at the center of that ventricle. When other points are stimulated the resulting initial deflection is upward in some leads, and this deflection has been shown to arise from excitation of the right ventricle (3).

The suggestion arising from these observations is that downward initial deflections produced by stimulation of the endocardial surface of the right ventricle might also have the same origin as the downward deflections induced by stimulation of the external surface, namely, excitation of the opposite ventricle. Other experiments on this topic throw no certain light on the subject (4, 5).

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² This paper was submitted with the original electrocardiograms as evidence of the results obtained. As a measure to conserve space and material the authors were requested to substitute for them a single figure (fig. 1) in which the results are illustrated by means of line drawings. These drawings follow as closely as possible measurements taken from actual electrocardiograms.

METHODS. Thirteen dogs were employed, prepared as previously described (6). An electrode holder was made to hold in juxtaposition two dipolar electrodes. One limb was inserted into the ventricle through the auricle and the other limb was applied directly opposite it on the external surface of the heart. By means of a thyatron stimulator, connected to the electrodes through a commutator, extrasystoles could be elicited from points directly opposite each other on the endocardium or the epicardium. Both the right and the left ventricle were explored. Electrocardiograms were taken from leads I and III, the lungs being fully expanded and the edges of the skin and subjacent muscles approximated with clips.

RESULTS. *A. Regions in which initial deflections of endocardial and epicardial extrasystoles were in same direction.* The first result of these experiments was the finding that in most regions of the heart stimulation of the endocardium and the overlying epicardium yielded complexes in which the initial deflections were not oppositely directed as Lewis found, but were the same. These complexes varied only in that the QRS interval of internally excited extrasystoles from the left ventricle was less than QRS in externally elicited extrasystoles from the same ventricle.

When the center of the right ventricle was stimulated the initial deflection was, as usual (7), upward in leads I and III when the stimulus was externally applied (fig. 1, 1 out). It was also upward when the endocardium was stimulated (fig. 1, 1 in). When the center of the left ventricle was stimulated, extrasystoles from the outside showed downward initial deflections in leads I and III (fig. 1, 3 out) (7). Extrasystoles elicited from the endocardium showed also a downward initial deflection (fig. 1, 3 in).

When points along the anterior septum were stimulated externally, lead I showed a downward initial deflection, while lead III showed an upward initial deflection (7). When subjacent points were stimulated on the interior of the heart, either in the right ventricle or in the left ventricle, the initial deflections of the resulting complexes were similarly downward in lead I and upward in lead III. The direction of the initial deflections was therefore the same, whether the endocardium or the epicardium was stimulated (fig. 1, 2 in and out).

Complexes elicited by stimulating the endocardium of the right ventricle and the overlying epicardium at the posterior septum were also identical with regard to the direction of initial deflections (fig. 1, 4 out and in). Because of technical difficulties, the endocardium of the left ventricle at the posterior septum was not stimulated.

Epicardial and subjacent endocardial stimulation of numerous points over the entire anterior right ventricle gave extrasystoles in which the initial complexes were also in the same direction. The configuration of extrasystoles elicited by stimulation of the epicardium in this region has already been described (2). In lead III there is no change as the electrode is moved laterally from the anterior septum. In lead I there is a change from the downwardly directed initial complex characteristic of an anterior septal extrasystole to the extrasystole produced by stimulation of the center of the right ventricle, in which the initial complex is directed upward. In this transition the initial downward deflection becomes smaller and an upward deflection appears following it, which increases in ampli-

tude as the point of stimulation approaches the center. The complex thus appears to have a Q of diminishing amplitude and an R wave of increasing amplitude. The same sequence was found when subjacent points on the endocardium were stimulated, and no points were found at which the initial deflections of epicardial and endocardial extrasystoles were oppositely directed. The initial deflections in lead III were also similarly directed in endocardial and epicardial extrasystoles, although of course in this lead there was no change in configuration with change in the position of the electrode (fig. 1, c and d, out and in).

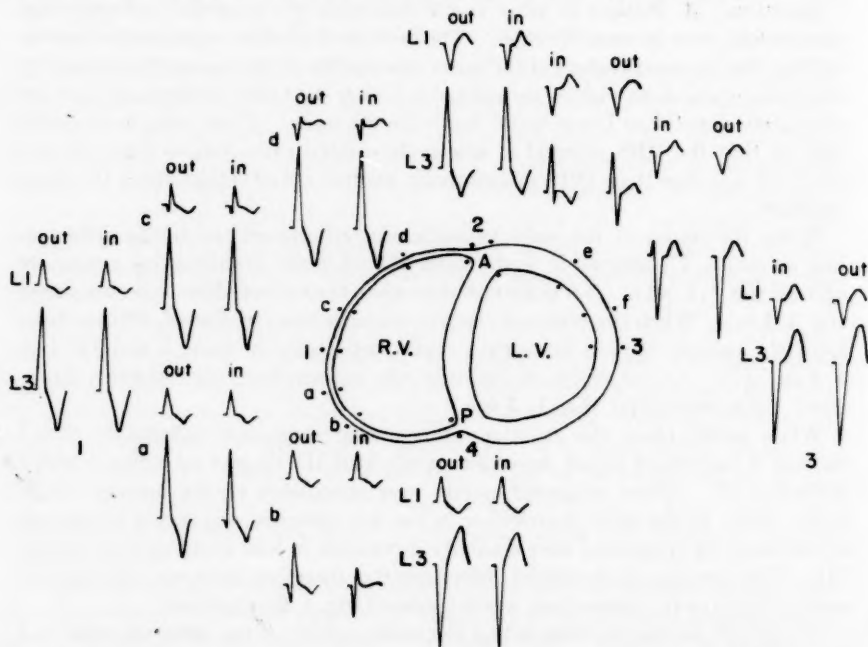


Fig. 1. A semischematic summary of the configuration in the electrocardiogram of extrasystoles elicited from epicardial and endocardial points. See text for explanation. The relations here depicted hold over a broad belt reaching from the base nearly to the apex.

B. Regions from which extrasystoles with divergent initial complexes could be obtained. (i) *Anterior left ventricle.* In four experiments the anterior left ventricle was thoroughly explored. The paired electrodes were moved progressively from the center of the left ventricle anteriorly toward the septum. At the center of the left ventricle initial complexes of both endocardial and epicardial extrasystoles were directed downward in both leads I and III (fig. 1, 3). As the electrode was moved toward the anterior septum, and at the septum, lead I remained unchanged both in endocardial and epicardial extrasystoles, which showed an initial downward deflection (fig. 1, e and f, lead I). In lead III extrasystoles elicited by epicardial stimulation of points between the center of the

left ventricle and the anterior septum showed the development of a small preliminary upward deflection (an R wave) which increased in amplitude as the downward deflection (S) decreased, until at the anterior septum only an R wave could be found (3). The extrasystoles elicited from subjacent points on the endocardium showed a similar transition in the development of an R wave. There was, however, one significant difference, namely, that the first appearance of an R wave occurred nearer the center than in the case of epicardial extrasystoles. It was therefore possible to find points in the anterolateral region of the left ventricle in which, in lead III, an R wave was present in the endocardial extrasystole, while there was none in the epicardial extrasystole (fig. 1, f, lead III). The area from which such divergent complexes could be obtained was considerably greater than that on the right ventricle. These complexes form a striking counterpart to the figure published by Lewis for the right ventricle.

Having found, in earlier experiments, that similar upward initial deflections in extrasystoles derived from epicardial stimulation of the left ventricle are produced by excitation of the right ventricle (3), and noting that stimulation of the inside and outside varied only in that the area yielding purely downward complexes was more restricted in the endocardium, it was postulated that the upward deflections of these extrasystoles were also due to excitation of the right ventricle. The anterior right ventricle was accordingly cooled by means of the thermal chamber previously employed (3) (fig. 2, 1 B) while extrasystoles were being elicited which showed in controls such upward initial complexes. The R wave in the normal complexes was reduced in magnitude and the S wave increased. Similarly the R wave of extrasystoles excited by stimulation of the endocardium was reduced greatly, while the S wave was increased (fig. 2, 1 B). Figure 2, 1 C shows the return to normal of both the normal complex and the extrasystole after cooling was discontinued and the heart was allowed to regain its previous temperature.

(ii) *Posterior right ventricle.* In three experiments it was possible to reproduce exactly the configurations described by Lewis for the right ventricle. Figure 1, 4, b, a, 1 shows the changing pattern in lead III of extrasystoles elicited from the epicardium (out) and the endocardium (in) at four points progressing from the posterior septum to the center of the right ventricle. In both "out" and "in" is shown a progression from a complex with downward initial deflection to a complex in which the initial deflection is upward. In both epicardial and endocardial extrasystoles this progression includes complexes having Q waves. The only difference is that the area in the endocardium from which purely upward initial deflections were obtained was more restricted than the area on the epicardium which yielded similar complexes, so that a region exists (fig. 1, a) where in lead III a small Q is present in the endocardial but not in the epicardial extrasystole. In lead I all these extrasystoles remained the same, showing only an upright initial complex or R wave, and no differences were found in the direction of epicardial and endocardial extrasystoles.

It has already been demonstrated that Q waves seen in lead III of extrasystoles elicited by stimulating the epicardium of the posterior right ventricle have their

origin in excitation of the posterior surface of the left ventricle (2). To test whether the similar Q waves in extrasystoles derived from the endocardium in the same regions have the same origin, the posterior left ventricle was heated and cooled while extrasystoles having Q waves were elicited by stimulation of the endocardium in an appropriate region of the posterior right ventricle. The virtual disappearance of the Q wave of such extrasystoles when the left ventricle was cooled (fig. 2, 2 B) and its return when the left ventricle was subsequently

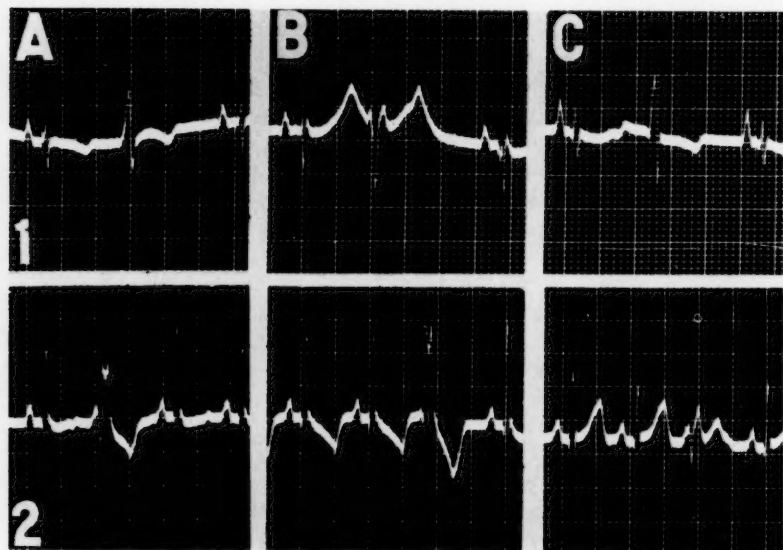


Fig. 2. 1 A, B, C. Nov. 19, 1942. 6.0 kgm. dog. Lead III. 1 A. Shows an extrasystole elicited by stimulating the left ventricular endocardium at a point near the anterior septum. 1 B. Shows great reduction in amplitude of R of the extrasystole by cooling the anterior surface of the right ventricle. 1 C. Shows return of R in the extrasystole when the right anterior ventricular surface returns to the normal temperature. 2 A, B, C. Nov. 24, 1942. 9.5 kgm. dog. Lead III. 2 A. Shows a Q in an extrasystole elicited by stimulating the right ventricular endocardium at a point midway between the lateral margin and the posterior septum. 2 B. Elimination of Q of extrasystole by cooling the posterior surface of the left ventricle. 2 C. Return of Q in the extrasystole by warming the surface of the posterior left ventricle.

warmed (fig. 2, 2 C) indicate that the Q wave of endocardial extrasystoles originates in the left ventricle just as does the Q wave of epicardial extrasystoles.

DISCUSSION. In these experiments only two regions of the ventricles were found in which there is divergence in the direction of the initial deflection of extrasystoles elicited from the endocardium and overlying epicardium. These are areas in the posterior part of the right ventricle near the lateral margin of the heart, and in the anterior portion of the left ventricle, also near the lateral border. Here there is divergence only in lead III, and conformity in lead I. In all other regions of the heart studied no divergence was found in the direction of the initial

deflections of extrasystoles from endocardial and overlying epicardial points in either lead I or lead III.

The divergence in right ventricular extrasystoles consists in the appearance of a small Q wave in endocardial extrasystoles while epicardial extrasystoles still show only an R wave. This appearance of a Q wave in endocardial extrasystoles is part of a transition in lead III from complexes elicited at the lateral margin, which show only an R wave, to complexes elicited at the posterior septal region, in which R has been completely replaced by a Q wave. This transition is the same as that undergone by epicardial extrasystoles, the only difference being that a Q wave appears in epicardial extrasystoles at points a few millimeters nearer the posterior septum than those in the endocardium which first show Q waves. Stated in another way, the region in the center of the right ventricle which on stimulation gives exclusively upright initial complexes is more restricted in the endocardium than on the epicardium.

The region at the center of the left ventricle which on stimulation gives exclusively downward initial complexes is also less extensive in the endocardium than on the epicardium. The transition from a purely downward complex in lead III to a purely upward complex in that lead, which takes place as the point of stimulation is moved from the center of the left ventricle to the anterior septum, therefore began nearer the center in endocardial extrasystoles. There is thus a region near the anterior left lateral margin in which the endocardial extrasystole shows a small R while the initial complex of the epicardial extrasystole is still exclusively downward.

The appearance and progressive increase in amplitude of a Q wave as points of stimulation on the epicardium of the right ventricle approached the septum suggested that the Q wave was derived from the excitation of the left ventricle. This concept of the origin of Q was proved by the readiness with which this wave could be increased or decreased or abolished by appropriate treatment of the left ventricle (2). In the present experiments the similarity of progression of the Q wave in endocardial extrasystoles from the right ventricle suggested that these Q waves also had their origin in the left ventricle. The fact that heating and cooling the left ventricle caused important modifications in the Q wave of such endocardial extrasystoles confirms this view. These experiments offer no support to the view that such Q waves arise from conduction of an impulse from endocardium to overlying epicardium in the right ventricle.

In a similar way it was demonstrated that the appearance and progressive increase in amplitude of an R wave in lead III, when points on the endocardium of the left ventricle were stimulated, was due to the progressively earlier activation of the right ventricle. Heating and cooling of the right ventricle modified the R wave of endocardial left ventricular extrasystoles just as they modified epicardial extrasystoles (3). Here again results are inconsistent with an explanation of the R wave on the basis of its origin in the left ventricle in an impulse travelling from endocardium to epicardium.

It was observed frequently that the QRS interval of left endocardial ventricular extrasystoles was definitely shorter than that of corresponding epicardial

extrasystoles. This indicates that conduction to the opposite ventricle takes place more rapidly when the endocardium is stimulated than when the extrasystole arises in the epicardium. If this be so, it can be understood why points were found on the endocardium near the lateral border of the left ventricle from which extrasystoles were obtained showing small R waves, while extrasystoles from overlying points on the epicardium do not show R waves. Divergence in the direction of the initial deflection of right ventricular extrasystoles from endocardium and epicardium must also arise from a slightly more rapid spread of excitation to the left ventricle when the endocardium of the right ventricle is stimulated than when an overlying epicardial point is the locus of stimulation.

It is to be remembered that in Lewis' experiments standard indirect leads were not employed, but that leads were placed on opposite sides of the chest, in line with the electrodes, to create optimum conditions for recording a limited potential arising in the endocardium. The situation is somewhat different in the present experiments. Lack of conformity in initial deflections of epicardial and endocardial extrasystoles was not found in regions which are lined up with the external leads. Thus lead III is known to be in line with the centers of the anterior right ventricle and the posterior left ventricle (8). The areas showing divergence in this lead were, however, found in the posterolateral region of the right ventricle and the anterolateral region of the left ventricle. These latter regions are in line with lead I, which failed to show any divergence between endocardial and epicardial extrasystoles.

SUMMARY

1. The configuration, in leads I and III of the electrocardiogram, of ventricular extrasystoles elicited by stimulation of points on the endocardium and the immediately opposite epicardium has been studied.

2. The direction of initial deflections of endocardial and epicardial extrasystoles was the same in both leads I and III over most of the surface of the heart. These regions included (1) the area over the septum, both anterior and posterior; (2) an area on the lateral margin of each ventricle, which is referred to as the center of the ventricle because it is equidistant from the septal margins, and (3) the entire anterior surface of the right ventricle.

3. The direction of initial deflections in lead I of the epicardial and endocardial extrasystoles was also found to be the same over the entire posterior portion of the right ventricle and the anterior part of the left ventricle.

4. The direction of initial deflections in lead III of epicardial and endocardial extrasystoles was the same over much of the posterior portion of the right ventricle and the anterior part of the left ventricle.

5. Two areas were found, of the anterior left ventricle toward the left lateral border and the posterior right ventricle toward the right lateral border, where the initial deflections of endocardial extrasystoles were opposite to those of extrasystoles from immediately overlying epicardial points.

6. These oppositely directed initial complexes of extrasystoles elicited from

such regions of the endocardium were shown to arise, as do the similar complexes from stimulation of near-by epicardial points, from excitation of the opposite ventricle.

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A RENAL REABSORPTIVE MECHANISM IN THE DOG COMMON TO GLYCIN AND CREATINE

ROBERT F. PITTS

From the Department of Physiology, Cornell University Medical College, New York City

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The development of an extensive glomerular filtering bed in the mammalian kidney has necessitated the parallel development of a similarly extensive tubular reabsorptive surface. The salvaging of water, salts, glucose, amino acids and other constituents of value from the glomerular filtrate constitutes a problem of homeostasis fully as important as that of excretion of waste materials. Quantitatively, reabsorptive processes outweigh purely excretory ones.

The presence of amino acids in the circulating plasma in appreciable concentrations and their almost complete absence from the urine under normal conditions point to an efficient renal mechanism for the reabsorption of these substances. Studies that have been made of excretion following administration of amino acids have not been such as to contribute to our knowledge of the kinetics of the reabsorptive system (Kirk, 1936; Doty, 1941). The importance of amino acids in the general economy of the body lends interest to such a study, while recent advances in therapy involving the parenteral administration of amino acids add an element of practical importance (Elman, 1937).

Experiments designed to outline the characteristics of the reabsorptive system in the dog for the amino acid glycine are presented in this paper. It has been found that glycine is reabsorbed from the glomerular filtrate by a stable active chemical transport mechanism which exhibits, as does the glucose reabsorptive system (Shannon and Fisher, 1938) limitation of transfer capacity. Creatine, which may be considered as a substituted glycine, is reabsorbed by the same mechanism. Hence, when either or both of these substances are presented to the renal tubules in increased amounts, they compete for the common reabsorptive system.

EXPERIMENTAL PROCEDURE. Observations have been made on four well trained female mongrel dogs. During the experiments they were loosely restrained on a comfortable animal board. Urine collections were made with the use of an indwelling catheter. Collection periods were 10 minutes in length, and if the urine flow amounted to less than 10 cc. per minute, the bladder was washed out and the washings added to the original urine. Since peripheral tissues may absorb large amounts of amino acids (Van Slyke and Meyer, 1913) leading to significant arterio-venous differences, all analyses were performed on arterial plasma. Arterial blood sampling at the exact mid-point of each urine collection period was greatly facilitated by the use of an indwelling femoral arterial needle fitted with a tight stylet. Constant creatinine and varying amino nitrogen concentrations in the plasma were obtained by infusions administered through the saphenous vein by means of a motor driven pump. Water, in amounts of 50 cc. per kgm., was administered per os 60 minutes prior to the start of the experiment.

Observations have been made with both rising and falling plasma concentrations of amino nitrogen. During any given period of observation, however, change in concentration has been restricted. This has been accomplished by administering infusions of various concentrations at constant rate for a period of 20 minutes prior to the start of experimental periods to establish approximate equilibrium. In those experiments in which high plasma levels were attained initially, an appropriate priming dose was given before starting the infusion. The slow rate of change of plasma concentration coupled with uniformly high rates of urine flow render dead space errors negligible.

Chemical methods. For amino acid determination plasma and diluted urines were treated with dialyzed, purified urease and a 1:6 picric acid filtrate was prepared. The ninhydrin gasometric determination of Van Slyke, Dillon, MacFadyen and Hamilton (1941) as modified by Hamilton (1942) was applied to the urea-free filtrate. Creatinine, creatine and glucose analyses were performed on iron filtrates of plasma (Steiner, Urban and West, 1932) and on diluted urines. Creatinine analyses were performed by the Folin and Wu (1919) method and creatine by the acid hydrolysis method as modified by Pitts (1934). The colors were read on an Evelyn colorimeter exactly 10 minutes after the addition of alkaline picrate to each tube. Glucose was determined by the Folin (1929) method as modified by Shannon, Farber and Troast (1941). Para-amino-hippuric acid was determined on cadmium filtrates (Fujita and Iwatake, 1931) by the method of Finkelstein, Aliminos and Smith (1941). Urine pH measurements were made in several experiments using a glass electrode without precaution to prevent escape of carbon dioxide. All analyses were performed in duplicate and if adequate checks were not obtained additional duplicates were run.

RESULTS. *The basis for measurement of amino nitrogen reabsorption.* The creatinine clearance has been used as a measure of glomerular filtration rate under conditions favorable for maximum accuracy, namely, constant plasma concentrations between 30 and 40 mgm. per 100 cc. The amount of amino nitrogen filtered at the glomeruli per unit of time is calculated as the product of the plasma concentration in milligrams per cubic centimeter and the rate of glomerular filtration in cubic centimeters per minute. The amount excreted is equal to the product of urinary concentration in milligrams per cubic centimeter and urine flow in cubic centimeters per minute. The amount reabsorbed is obviously the difference between these two quantities.

The characteristics of the reabsorptive system for glycine amino nitrogen. The essential data from 3 experiments on dog 1 are given in table 1. It may be seen by referring to experiment 12 of this table that normally more than 98 per cent of filtered amino nitrogen is reabsorbed. As the plasma concentration is raised by the infusion of glycine the amount reabsorbed fails to increase in proportion to the amount filtered and excretion becomes appreciable. Both excreted and reabsorbed moieties continue to increase until the latter reaches a limiting value of approximately 21 mgm. per minute. Further increases in the amount filtered are accompanied by proportionate increases in the amount excreted. When the amount filtered exceeds that amount necessary to saturate the reabsorptive

TABLE 1

Experiments on a normal dog which show the relationship between the amount of glycine amino nitrogen filtered and the amounts reabsorbed and excreted

All infusions at a rate of 5 cc. per minute. Dog 1; 18.7 kgm; S.A. 0.72 sq.m.

EXPER. NO.	GLOMERULAR FILTRATION RATE	URINE FLOW	AMINO NITROGEN					CLEARANCE RATIO AMINO-N CREATININE
			Plasma conc.	Urine conc.	Filtered	Excreted	Re- absorbed	
Infusion 0% glycine								
12	cc./min.	cc./min.	mgm. %	mgm. %	mgm./min.	mgm./min.	mgm./min.	
	82.1	9.85	3.76	0.50	3.09	0.05	3.04	0.02
	85.2	9.80	3.65	0.67	3.11	0.07	3.04	0.02
Infusion 2% glycine								
	94.2	3.80	8.25	3.16	7.77	0.12	7.65	0.02
	97.2	4.40	9.25	7.90	9.00	0.35	8.65	0.04
Infusion 3% glycine								
	109	5.30	12.2	37.6	13.3	2.00	11.3	0.15
	107	5.20	13.2	46.4	14.1	2.41	11.7	0.17
Infusion 4% glycine								
	110	5.20	17.5	84.3	19.3	4.39	14.9	0.23
	109	5.30	19.0	96.3	20.7	5.10	15.6	0.25
Infusion 6% glycine								
7	123	5.70	27.4	234	33.7	13.4	20.3	0.40
	118	5.45	27.2	235	32.1	12.8	19.3	0.40
	120	4.90	27.2	252	32.7	12.4	20.3	0.38
Infusion 3% glycine								
	118	3.50	23.7	245	28.0	8.58	19.4	0.31
	114	3.30	22.4	224	25.6	7.39	18.2	0.29
	115	3.35	20.8	197	24.0	6.60	17.4	0.28
	116	3.05	20.2	188	23.5	5.74	17.8	0.25
	112	2.90	19.7	185	22.1	5.36	16.7	0.24
Infusion 10% glycine								
8	109	12.1	47.7	250	52.0	30.2	21.8	0.58
	100	10.4	49.5	278	49.5	28.9	20.6	0.58
	95.5	9.30	52.3	309	50.0	28.7	21.3	0.58
	99.3	9.40	52.8	324	52.5	30.4	22.1	0.58
Infusion 5% glycine								
	95.8	6.20	46.5	375	44.5	23.3	21.2	0.52
	95.8	5.50	43.5	377	41.6	20.8	20.8	0.50
	97.5	5.35	41.5	365	40.5	19.5	21.0	0.51
	94.2	4.90	40.0	360	37.7	17.7	20.0	0.47

system (between 30 and 35 mgm. per minute filtered) either gradual increase or gradual decrease of plasma concentration is without effect on the quantity reabsorbed (expt. 8).

All of our observations on dogs 1 and 2 are plotted in figures 1 and 2. Less extensive observations on two other dogs are in complete qualitative agreement. These figures illustrate well the gradual approach to a maximal rate of reabsorption for glycine amino nitrogen (T_m) and the consequent gradual increase in excretion. These characteristics serve to distinguish sharply the amino nitrogen reabsorptive mechanism from the glucose system. According to Shannon and Fisher (1938), glucose reabsorption keeps pace with filtration until the maximal

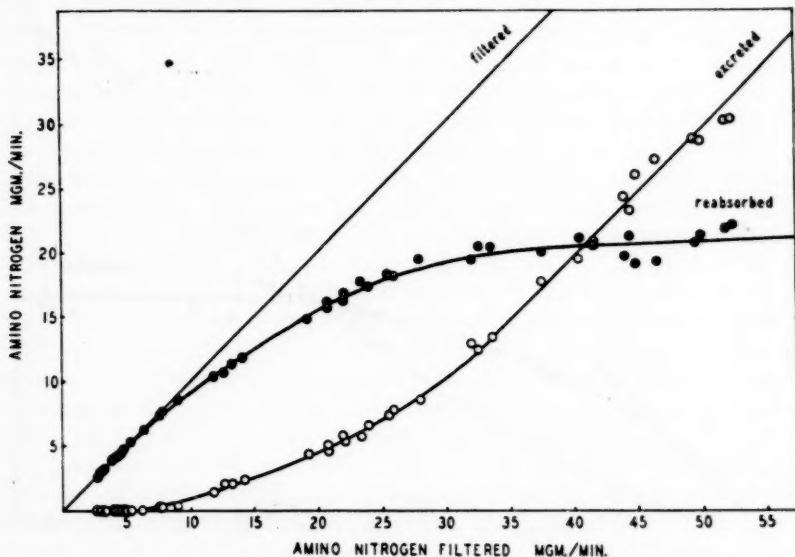


Fig. 1. The renal reabsorption and excretion of glycine amino nitrogen as a function of the quantity filtered in dog 1.

rate of reabsorption is attained. Excretion then begins and increases exactly in proportion to the excess filtered.

The amino nitrogen reabsorptive system appears to be no less stable and reproducible than the glucose system. The experiments shown in figures 1 and 2 were carried out over a period of 3 months and the limited scatter of the data is evidence of stability of the mechanism. The maximal reabsorptive capacity for glycine amino nitrogen, however, is characteristic for a given animal. For four dogs the values are 13, 17, 21 and 23 mgm. per minute. All dogs were between 16 and 19 kgm. in weight with surface areas of about 0.7 sq.m. Thus the maximal reabsorptive capacity in mongrel dogs is not a simple function of surface area or body weight. The reabsorption of amino nitrogen was in no way conditioned in these experiments by the reaction of the urine, for pH measurements showed only

negligible variations within limits of pH 6.3 and pH 7.2, and seemed more correlated with urine volume than with amino acid content.

Competition between glycine and creatine for a common reabsorptive system. Tubular reabsorption of creatine is indicated by its absence from the urine under normal conditions and by the progressive increase in clearance as the plasma creatine concentration is raised (Pitts, 1934). Recalculation of the data presented in that paper shows that at plasma concentrations up to 100 mgm. per 100 cc., the quantity reabsorbed still increases in the face of marked excretion. This superficial resemblance of the reabsorptive processes for creatine and glycine and the fact that, chemically, creatine may be considered as a substituted methyl glycine, suggested the possibility that a single tubular transfer mechanism might

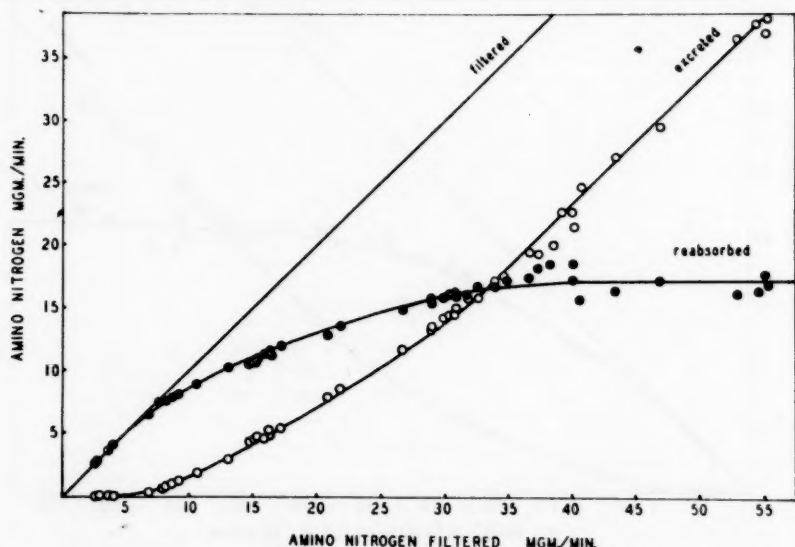


Fig. 2. The renal reabsorption and excretion of glycine amino nitrogen as a function of the quantity filtered in dog 2.

be common to the two substances. The experiments presented in table 2 on dog 3 indicate that the prediction is correct. In experiment 21, the concentration of creatine in the plasma was raised to such a level that accurate analyses could be made in the presence of creatinine. In the first two periods, at normal plasma amino nitrogen levels, 2.7 mgm. of creatine were reabsorbed per minute. As plasma amino nitrogen concentrations were raised by progressively increasing the glycine content of the infusions, the amount of creatine reabsorbed diminished. In the last two periods of experiment 21, upon saturation of the tubular transfer system with amino nitrogen, creatine reabsorption dropped to zero. In experiment 22 the infusion of larger amounts of creatine approximately doubled the quantity reabsorbed. Again reabsorption dropped to zero on administering large

amounts of glycine. These experiments indicate that glycine and exogenous creatine compete for a common reabsorptive system. Experiment 23 duplicates experiment 22 as nearly as possible except that both creatine and creatinine were omitted from the infusions. Creatinine was omitted in order to analyze, with some fair degree of accuracy, the endogenous apparent creatine of plasma. Since the glomerular filtration rate was unknown, amounts filtered and reabsorbed could not be calculated. However, an increase in the amount excreted from 0.008 mgm. per minute in the control periods to 0.251 mgm. per minute after glycine, is indicative of the fact that glycine and endogenous apparent creatine are reabsorbed by the same mechanism. These experiments should well disturb those groups of investigators who study precursors of creatine by feeding various amino acids and note only the elimination of excess creatine in the urine. In fact the use of such a method of approach in any metabolic study cannot be too heartily condemned, for it neglects possible direct alterations of renal function.

The corollary of experiment 23, namely, increasing plasma creatine to high levels and studying normal amino nitrogen excretion has been performed with completely negative results. This is interpreted by us as suggesting a much greater affinity of amino nitrogen than of creatine for some common link of the reabsorptive chain. It is not unreasonable to presume a rather low affinity for creatine in view of the relatively insignificant amounts reabsorbed at even the highest plasma levels.

Alterations in renal function produced by glycine. Claims of acute renal damage from the administration of large amounts of amino acids have been made (Newburgh and Marsh, 1925). Since our animals are all alive, we have had no opportunity to examine the kidneys microscopically. Functionally, however, neither acute damage as evidenced by hematuria, nor chronic damage as evidenced by reduced glomerular filtration rate or tubular reabsorptive capacity have been observed. We have performed some 16 experiments on dog 1, during the course of which a total of over one kilogram of various amino acids has been administered, without evidence of any chronic reduction of renal function. The reduction in reabsorption of creatine shown in table 2 cannot be assigned to non-specific renal damage for it is manifest in periods 3 and 4 of experiment 21 at plasma amino nitrogen concentrations within a range attainable after a meat meal.

However, significant functional variations in glomerular filtration rate and minimum effective renal plasma flow are routinely observed on administration of glycine, as is evident on inspection of table 3. The feeding of meat, casein and glycine or the parenteral administration of the latter substance increases the rate of glomerular filtration in the dog (Pitts, 1935). Hiatt (1942) has shown that meat feeding in the seal causes an even greater increase in glomerular function and a marked increase in renal plasma flow. The p-amino hippuric acid clearance given in table 3 is accepted as a measure of minimum effective renal plasma flow (Finkelstein, Aliminos and Smith, 1941). It may be seen that elevation of plasma amino nitrogen by infusion of glycine is followed by progressive increase in glomerular filtration rate and renal plasma flow. At moderately elevated plasma

TABLE 2

Experiments on a normal dog which illustrate competition between creatine and glycine amino nitrogen for a common reabsorptive mechanism

All infusions at a rate of 5 cc. per minute. Dog 3; 17.5 kgm; S.A. 0.76 sq.m.

EXPER. NO.	GLOMERULAR FILTRATION RATE	AMINO NITROGEN			CREATINE		
		Plasma conc.	Excreted	Reabsorbed	Plasma conc.	Excreted	Reabsorbed
Infusion 0.5% creatine; 0% glycine							
21	cc./min.	mgm. %	mgm./min.	mgm./min.	mgm. %	mgm./min.	mgm./min.
	74.0	5.50	0.04	4.04	31.9	20.9	2.7
	74.8	5.50	0.06	4.02	29.5	19.4	2.7
							2.7
Infusion 0.5% creatine; 2% glycine							
	79.2	9.11	0.60	6.62	26.5	19.2	1.8
	77.6	10.2	0.94	6.97	25.5	18.2	1.6
							1.7
Infusion 0.5% creatine; 4% glycine							
	74.4	15.6	2.99	8.61	22.0	15.7	0.7
	72.0	18.2	3.67	9.45	22.7	15.5	0.9
							0.8
Infusion 0.5% creatine; 6% glycine							
	66.0	31.0	8.30	12.2	23.1	15.5	0.0
	67.6	36.4	10.4	14.2	23.5	15.9	0.0
							0.0
Infusion 2.0% creatine; 0% glycine							
22	59.7	4.17	0.02	2.47	109	59.9	5.2
	64.2	4.03	0.02	2.57	108	63.6	5.9
	64.8	3.66	0.02	2.35	112	66.3	6.3
							5.8
Infusion 2.0% creatine; 7.5% glycine							
	62.6	47.1	17.1	12.4	114	71.4	0.0
	54.5	55.4	18.1	12.1	118	65.2	0.0
	51.7	63.3	19.4	13.4	123	64.1	0.0
							0.0
Infusion 0% creatine; 0% glycine							
23		3.72	0.02		0.84	0.006	
		3.64	0.02		0.82	0.010	
		3.51	0.03		0.80	0.008	
						0.008	
Infusion 0% creatine; 7.5% glycine							
		46.1	17.1		0.98	0.232	
		53.2	19.4		1.02	0.250	
		59.8	22.4		1.06	0.271	
						0.251	

levels, renal plasma flow increased disproportionately to glomerular filtration rate so that filtration fraction decreases (ratio of creatinine to p-amino-hippuric acid clearance). At still higher levels filtration fraction returns to the usual figure of about 0.30.

TABLE 3

Experiments on a normal dog which indicate the types of renal functional change which result from glycine infusion

All infusions at a rate of 5 cc. per minute. Dog 2; 18.7 kgm.; S.A. 0.71 sq.m.

EXPER. NO.	GLOMERULAR FILTRATION RATE	URINE FLOW	PLASMA CONCENTRATION		CLEARANCE		AMINO NITROGEN	CLEARANCE RATIO	
			Amino nitrogen	p-Amino hippurate	Amino nitrogen	p-Amino hippurate	Re- absorbed	Amino-N Creatinine	Creatinine Hippurate
Infusion 0% glycine									
11	cc./min.	cc./min.	mgm. %	mgm. %	cc./min.	cc./min.	mgm./min.		
	63.4	8.10	4.19	3.12	0.72	210	2.63	0.01	0.30
	64.7	8.15	4.18	2.85	0.86	206	2.67	0.01	0.31
Infusion 2% glycine									
	74.5	6.90	9.08	2.20	3.30	275	6.46	0.04	0.27
	78.2	6.30	10.2	2.14	5.83	267	7.23	0.08	0.29
Infusion 4% glycine									
	81.0	8.20	25.6	1.60	31.2	341	12.8	0.38	0.24
	82.0	7.67	26.7	1.53	31.3	361	13.5	0.38	0.23
Infusion 6% glycine									
	82.5	9.40	35.1	1.70	38.0	300	15.5	0.46	0.27
	81.1	9.35	38.2	1.79	39.2	286	16.0	0.48	0.28
Infusion 9% glycine									
13	86.6	20.0	63.1	1.92	60.1	264	16.7	0.69	0.33
	85.1	18.0	65.0	1.92	57.2	262	18.0	0.67	0.33
	82.2	18.9	67.1	1.92	57.0	254	16.8	0.69	0.32
	75.4	16.9	70.0	1.95	52.4	244	16.2	0.70	0.31
Infusion 4.5% glycine									
	72.1	11.2	64.5	2.10	45.6	228	17.1	0.63	0.32
	69.2	10.1	62.7	2.19	43.1	218	16.4	0.62	0.32
	66.6	9.0	60.6	2.22	41.0	215	15.7	0.61	0.31
	69.0	9.9	57.8	2.26	39.2	222	17.2	0.56	0.31

It has been commonly observed that glomerular filtration rate tends to drop when plasma amino nitrogen is maintained elevated for long periods of time. This is evident in experiment 8 of table 1 and experiment 13 of table 3. Note also in experiment 13 that renal plasma flow diminishes. It is rather surprising that

filtration rate and blood flow are not more seriously depressed considering the severe signs of somatic and visceral disturbance. These signs include vomiting, dilatation and fixation of the pupils, weakness and muscular inco-ordination. Recovery is relatively rapid and essentially complete within a few hours of the end of the experiment. To date no fatalities have occurred as a result of amino acid infusion, although, as described in the next section, near fatalities have occurred.

Independence of amino nitrogen and glucose reabsorption. The reabsorptive systems for glycine and glucose are similar in so far as both show a limitation of maximal transfer capacity. They are different in the way that the limit is approached. To decide whether we are dealing with two distinct systems or with differences in the way two substances are handled by a common system, experiments were performed similar to those on creatine. Only to a degree have our experiments been successful. Saturation of the reabsorptive system with glucose has no effect on reabsorption of normal amino nitrogen. Saturation of the reabsorptive system with glycine amino nitrogen has no effect on reabsorption of normal glucose. Phlorizin in amounts sufficient to produce complete glycosuria does not increase the normal amino nitrogen excretion. These experiments are consonant with the view that the reabsorptive mechanisms are different. However, when the plasma concentrations of glucose and glycine were raised simultaneously to saturation levels, a serious collapse of filtration rate occurred. Associated with this collapse was an approximately equivalent reduction in both glucose and amino nitrogen reabsorptive capacities. Toxic manifestations of high plasma glucose and amino nitrogen were extreme, including coma and a rigidity of a decerebrate type. In one instance the animal was put aside for later autopsy, only to recover after a few hours with no residual signs of renal impairment. The depression of reabsorptive capacity in these experiments we attribute to circulatory collapse and complete closure of some glomeruli, with consequent reduction in the number of tubules contributing to the reabsorptive capacity of the kidney, and not to competition for a common reabsorptive system.

DISCUSSION. Shannon and Fisher (1938) have contributed a simple explanation of those cellular processes which impose a limitation on tubular reabsorption of glucose. With certain modifications, these concepts may be applied to reabsorption of glycine amino nitrogen. There is postulated a stable cellular component B , present in fixed amount, with which amino nitrogen A in the tubular fluid enters into combination in the course of reabsorption. The decomposition of of this compound AB to deliver A into the peritubular interstitial fluid is a first order process (fig. 3). If sufficient A is present to completely transform all of B into AB , the rate of transfer across the cell becomes constant and limited by the velocity of this reaction and by the amount of AB present. This concept explains equally well a maximal reabsorptive capacity for glycine and glucose. Of course substance B is different in the two systems.

Shannon and Fisher have assumed for glucose that this second reaction proceeds rather slowly in relation to the rate of attainment of equilibrium in the first reaction, namely, the combination of A with B to form AB . If the postulate

is reversed, namely, the second reaction proceeds rapidly in relation to the rate of attainment of equilibrium in the first, the gradual approach to a limiting tubular reabsorptive capacity for amino nitrogen finds ready explanation. Under conditions such that free B exists in the cell (incomplete saturation of the reabsorptive system), the amount transferred is limited by the effective rate of combination of A (glycin) with B to form AB . Assuming total B ($B + AB$) to be constant, rate of transport will depend upon the concentration of A and the specific velocity of its combination with B . The lower this velocity of combination the more gradually will the reabsorptive system be saturated. The higher this velocity

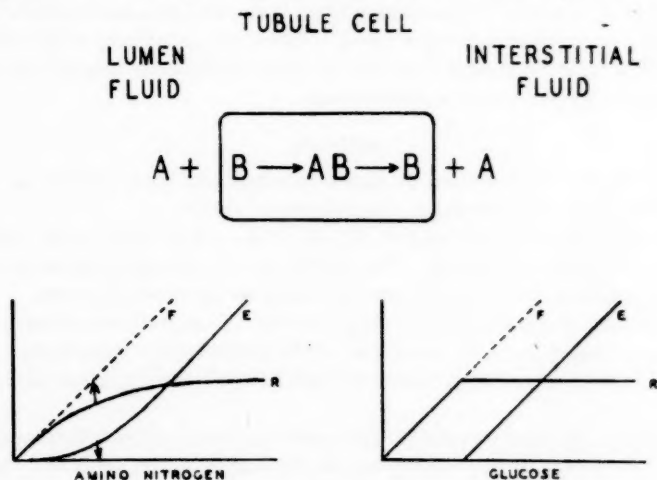


Fig. 3. A schematic representation of the cellular processes governing the reabsorption of amino nitrogen. B is a stable cellular component present in limited and fixed amount with which substance A (amino nitrogen) in the lumen fluid combines during reabsorptive transfer to the peritubular interstitial fluid. The properties of the system are discussed in the text.

the more nearly will the amino nitrogen system behave like the glucose system (note arrows in the inset graph for amino nitrogen in fig. 3).

Shannon (1938) has given an alternative explanation of what may be fundamentally a similar phenomenon, namely, the gradual approach to saturation of the reabsorptive system for the sugar xylose. Xylose and glucose are reabsorbed by a common cellular mechanism, yet the characteristics of the two processes are dissimilar in that xylose is excreted at all plasma concentrations and the amount reabsorbed appears to increase in proportion to concentration over a wide range. Shannon assumes that equilibrium conditions are maintained for xylose no less than for glucose. Differences in the two reabsorptive processes are then explicable in terms of differences in equilibrium constants for the combination of the two sugars with the common cellular element B . An equilibrium

constant for xylose, some 1500 times that for glucose would approximately account for the different characteristics of their reabsorptive processes. One might assume an intermediate equilibrium constant to account for a maximal reabsorptive capacity which is reached more gradually for glycine than for glucose and more rapidly for glycine than for xylose. We are however inclined toward the first explanation presented as a result of our further studies on the reabsorption of other amino acids to be presented subsequently.

The competition between creatine and glycine for a common reabsorptive mechanism depends upon the substance *B* common to both reabsorptive systems. If combined with glycine it is unavailable to creatine, and as a consequence creatine reabsorption is proportionately reduced. However, the affinity of glycine for this common link must be greater than that of creatine, for large amounts of creatine do not depress amino nitrogen reabsorption.

SUMMARY

1. The tubular reabsorption of amino nitrogen has been assessed at various arterial plasma levels obtained by the infusion of glycine.

2. Amino nitrogen is reabsorbed by an active mechanism which exhibits a limitation of transfer capacity. The maximal rate of reabsorption is attained rather gradually and as a consequence no sharp renal threshold exists.

3. Creatine is reabsorbed by the same system. Competition between creatine and amino nitrogen for a common link in the reabsorptive chain brings about a reduction in the amount of creatine reabsorbed at elevated plasma amino nitrogen levels.

4. As a result of this competition for a common renal reabsorptive mechanism, studies of creatine precursors based on the feeding of amino acids and the measurement of excess creatine eliminated in the urine are rendered suspect.

5. While the administration of large amounts of glycine produces marked immediate alterations in renal function, we have seen no evidence of any chronic damage.

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RAPID ACCLIMATIZATION TO WORK IN HOT CLIMATES

SID ROBINSON, E. S. TURRELL, H. S. BELDING AND S. M. HORVATH

From the Fatigue Laboratory, Harvard University¹, Boston

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The problem of man's acclimatization to heat has claimed the attention of numerous physiologists. There seems to be general agreement about some of the changes that take place, although the extent of these changes and the reasons for them are less certain. Almost all observers agree that working men in hot climates have higher blood volumes and interstitial fluid volumes than the same men in cold climates. Forbes, Dill and Hall (1940) found this change to be slight; Bazett et al. (1940) found it to be great and to come on early. Other changes associated with acclimatization to heat have been reported: Scott et al. (1940) noticed a temporary increase in resting cardiac output as well as an increase in peripheral circulation; Lee (1940) found a decline in heart rate, and Burton (1940) observed an initial increase in the heat exchange followed by a return to the control level. Many observations have been made upon the changes in the sweat in acclimatization to heat. Dill and his collaborators (1933, 1937, 1938) showed a decrease of 50 per cent in the concentration of sodium chloride in the sweat of working men, which decrease Johnson et al. (1943) have attributed to the lower body temperatures resulting from acclimatization. Associated with these changes are the obvious alterations in the total amount of sweat, the increase in water ingested and the decrease in the urine excretion, as noted by Adolph and Dill (1938). Most observers agree that the process of acclimatization results in an increased output of sweat under a given set of conditions—Haldane (1935), Moss (1923), Adolph and Dill (1938), Dill (1938) and Winslow et al. (1938)—in addition to the obvious increased sweating when the change is made from one climate to another. On the other hand, Knipping (1923) reported that sweating decreases with acclimatization—a discrepancy undoubtedly due to differences in the activities of the subjects.

In addition to these specific and easily measurable changes, certain others of a more general character are known to occur. The comfort zone of temperature is higher in summer than in winter (Yaglou, 1927) and with acclimatization a man's ability to do prolonged physical work in the heat improves markedly (Robinson et al., 1941). The present study represents an attempt to measure quantitatively this improvement in the ability to do work and particularly to follow its time relations. How quickly does the improvement occur and how soon is it lost when returning to a cooler environment? Some effort was made also to determine to what physiological variables this improvement is related.

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PROCEDURE. The experiments were carried out in Boston during February and March in an artificially heated room where the temperature was about 104°F. and the humidity 23 per cent. In no single experiment reported here on any given man did the average room temperature vary by more than 0.7°F. nor the humidity by more than 2 per cent from the mean for that man's entire series of experiments. The work consisted of walking on a motor driven treadmill at 3.5 m.p.h. on a grade of 5.6 per cent for four of the subjects and 4.0 per cent for the other man. With two exceptions all walks for each man were at the same speed and grade and for the same length of time, the duration being determined by the length of time required to exhaust him at the beginning. These walks will be referred to below as the standard hot room walks. The program was continued until the men had trained for 10 to 23 days. The exceptions to the standard walk were by subjects SR and WH who on one occasion after acclimatization continued walking at the standard grade and speed for three times the duration of their original walks. On another occasion these same two subjects walked on a higher grade (9 per cent) for their standard length of time. In addition to the work periods, subjects HB, ET, and SR spent an additional 3 to 4 hours each day in the hot room making observations on other subjects.

The heat produced by the body in the walk on a 5.6 per cent grade is about 30 per cent greater than the heat produced by men in the regular army march on the level with pack. The men used as subjects were five laboratory workers, all of whom were accustomed to walking on the treadmill under ordinary laboratory conditions but none of whom had been exposed to heat since the preceding summer. All of them were in such good condition that they completed a 40 mile hike on the road in one day. During all experiments included in this paper they wore standard army summer cotton trousers, shirt, tie, woolen socks and service shoes. On about half of their days of acclimatization, subjects HB, ET, and SR wore clothing which varied from this costume and the resulting data are not included in this paper. Pulse rate was determined by palpation, rectal temperature by clinical thermometer, and skin temperature by four thermocouples fixed on the skin, one each on chest, back, thigh and upper arm. The rate of water loss from skin and lungs was determined by weighing the nude subject before and after work. Metabolic weight loss was subtracted from the weight differences. Oxygen intake was determined once during each experiment by collecting and measuring the subjects' expired air and analyzing samples in the Haldane apparatus.

RESULTS. The effects of acclimatization on the heart rates, rectal temperatures, and skin temperatures of the men during the work experiments in the hot room are shown in figure 1. Except for ET, who walked on the lower grade, the men approached heat exhaustion in the early experiments, with high skin temperatures, rectal temperatures of 103° to 104°F., and heart rates averaging 178 beats per minute during the last 20 minutes of work. It is obvious from the data in figure 1 that repetitions of the constant task increased the comfort and ease with which the work could be done. It is significant that about 80 per cent of the improvement noted is found to have occurred in the first 7 days of exposure. After 23 days of acclimatization by walking in the hot room two men (SR and

HB), in order to determine the completeness and effectiveness of their acclimatization, repeated the standard walk with the room temperature lowered to 72°F. In the walks in the cooler room the men reached heat balance with their rectal

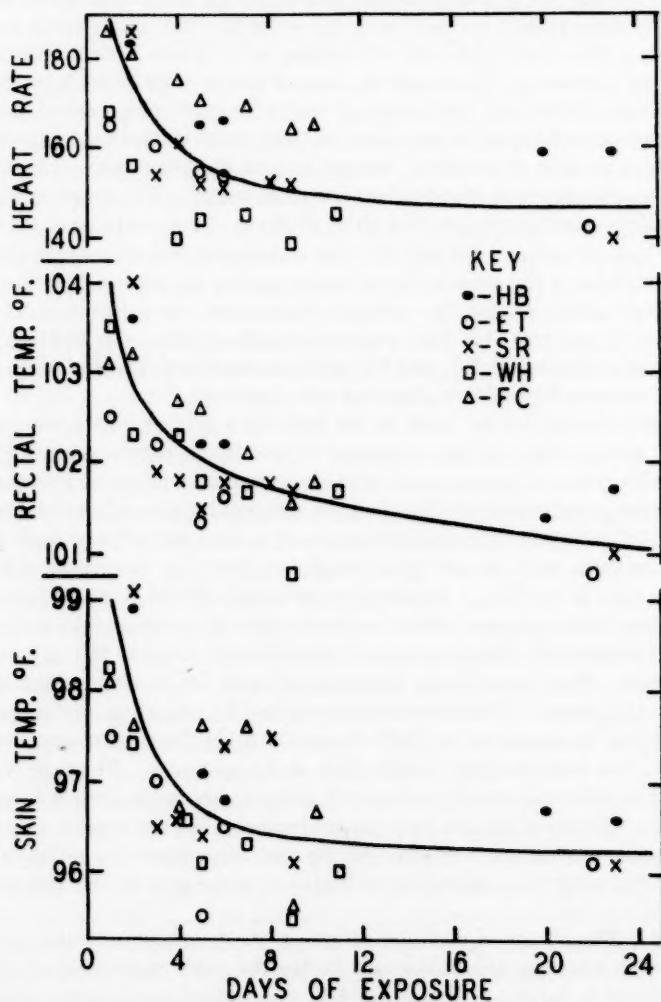


Fig. 1. Acclimatization to heat as shown by the lowering of body temperatures and heart rates of men in the standard work experiment (room temperature 104°F., humidity 23 per cent). On some of the days of exposure the men wore clothing which varied from the standard costume and the resulting data are omitted from this figure.

temperatures and metabolic rates the same as they were in their hot room experiments at the end of acclimatization (table 1). In the cool room experiments, radiation and convection played a large part in the dissipation of body heat, and,

therefore, loss of weight by evaporation from skin and lungs was small (table 1). In the hot room the men even absorbed heat from the room. Under these conditions, evaporation became great enough to dissipate all the heat produced within the body plus that absorbed by it (table 1). The fact that skin temperatures, rates of sweating, and heart rates were higher while the acclimatized men were working in the hot room experiments than when they were in the cool room shows that the mechanisms for heat dissipation were under greater stress in the

TABLE 1

Comparison of the performance of the standard work by men in the cool room and in the hot room after they were acclimatized to heat

SUBJECT	ROOM CONDITIONS		METABOLISM	BODY TEMP.		SWEAT	HEART RATE
	Temp.	Rel. Hum.		Rectal	Skin		
	^{°F.}	<i>per cent</i>	<i>Cal./hr.</i>	^{°F.}	^{°F.}	<i>kgm./hr.</i>	
SR	104.1	23	453	101.0	96.1	1.41	140
	72.1	32	450	100.8	87.5	0.58	128
HB	103.8	25	482	101.4	96.6	1.43	159
	71.9	32	485	101.3	87.8	0.61	134

TABLE 2

The effects of acclimatization on the metabolic rate and the rate of sweating of men in the standard work experiments

(Room temperature 104°F., relative humidity 23 per cent, rate of walking 3.5 m.p.h. on a 5.6 per cent grade)

SUBJECT	BODY SURFACE	BODY WEIGHT		METABOLISM		SWEAT	
		Before acclimatization	After acclimatization	Before acclimatization	After acclimatization	Before acclimatization	After acclimatization
	<i>m²</i>	<i>kgm.</i>	<i>kgm.</i>	<i>cal./hr.</i>	<i>cal./hr.</i>	<i>kgm./hr.</i>	<i>kgm./hr.</i>
HB.....	1.88	69.1	68.5	526	483	1.47	1.43
ET*.....	2.00	72.6	72.5	500	482	1.47	1.53
SR.....	1.75	65.4	64.3	540	453	1.40	1.41
WH.....	1.79	66.9	66.4	574	506	1.44	1.45
FC.....	2.00	84.9	86.2	686	686	1.35	1.57
Average....	1.88	71.8	71.6	565	522	1.43	1.48

* Subject ET walked on a 4 per cent grade as compared with a grade of 5.6 per cent for the other subjects.

hot room. (Nielsen (1938) has also observed the constancy of the rectal temperature of a man working at a constant rate in widely different environmental temperatures and has shown the adjustments to be due to evaporative and circulatory changes.)

During acclimatization subject FC experienced an increase in his rate of sweating in the standard work experiments but showed no significant change in his metabolic requirement for the work (table 2). The other four subjects were more

successful in becoming acclimatized than FC—they underwent decreases in metabolism yet showed no significant changes in their rates of sweating in the standard work experiments (table 2). The effect of acclimatization on the maximal capacity for sweating was determined by increasing the intensity of work in the hot room in one experiment on each of subjects SR and WH. Table 3 shows

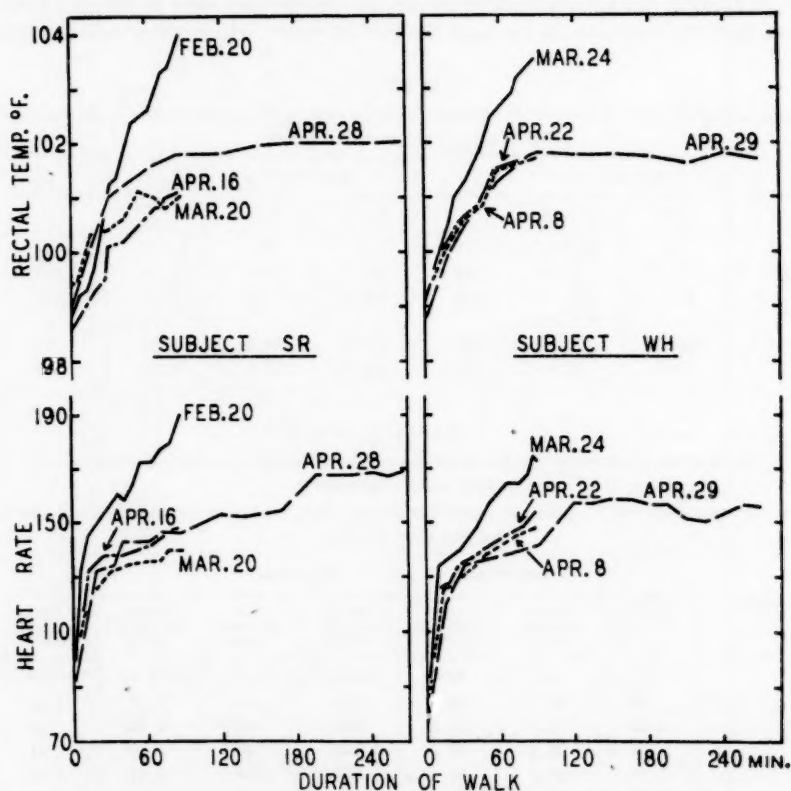


Fig. 2. Acclimatization to heat as shown by the lowering of body temperatures and heart rates of two men walking at 3.5 m.p.h. on a 5.6 per cent grade (room temperature 104°F., humidity 23 per cent).

a. Subject SR was acclimatized by 23 exposures between February 20 and March 20. After March 20 his only exposures were on April 16 and 28.

b. Subject WH was acclimatized by 11 exposures between March 24 and April 8. After April 8 his only exposures were on April 22 and 29.

that there was an average increase of 21 per cent in the actual capacities of these two men to sweat.

It is of practical importance to know how much more work men can do in the heat after acclimatization than they could before. Accordingly, after subjects WH and SR were acclimatized, tests were conducted in which they walked at the standard rate and grade in the hot room with ease for 4½ hours. This was three

times as long as in their original exhausting walks. It should be noted that no rest periods were taken in these long experiments. Figure 2 gives a comparison of the performances before and after acclimatization by these two men. Their rectal temperatures in the long walks were only moderately elevated and remained constant throughout the last 3 hours. Their heart rates never reached critical levels. On the other hand, in the original experiments they were completely exhausted in $1\frac{1}{2}$ hours with extremely high body temperatures and heart rates.

TABLE 3

The increase in the maximal rate of sweating with acclimatization

Before acclimatization maximal sweating was induced by the standard walk and after acclimatization by raising the intensity of the walk to 3.5 m.p.h. on a 9 per cent grade. (Room temperature 104°F ., relative humidity 23 per cent.)

SUBJECT	BEFORE ACCLIMATIZATION		AFTER ACCLIMATIZATION	
	Sweat	Rectal temp.	Sweat	Rectal temp.
	kgm./hr.	$^{\circ}\text{F}$.	kgm./hr.	$^{\circ}\text{F}$.
SR.....	1.40	104.0	1.85	103.0
WH.....	1.42	103.5	1.66	102.0
Average.....	1.41	103.8	1.76	102.5

TABLE 4

Loss of acclimatization to standard work in the hot room

The skin temperatures and heart rates represent the average of several determinations made during the last 20 minutes of each work experiment

SUBJ.	BEFORE ACCLIMATIZATION			AFTER ACCLIMATIZATION				LOSS OF ACCLIMATIZATION			
	Rectal temp.	Skin temp.	Heart rate	Period of acclim.	Rectal temp.	Skin temp.	Heart rate	After expos. ceased	Rectal temp.	Skin temp.	Heart rate
	$^{\circ}\text{F}$.	$^{\circ}\text{F}$.		days	$^{\circ}\text{F}$.	$^{\circ}\text{F}$.		days	$^{\circ}\text{F}$.	$^{\circ}\text{F}$.	
FC.....	103.1	98.1	186	10	101.8	96.7	164	15	102.9	97.6	176
HB.....	103.6	99.0	184	23	101.4	96.6	159	24	102.9	97.1	178
SR.....	104.0	99.1	186	23	101.0	96.1	140	26	101.2	96.1	146
ET.....	102.5	97.5	168	21	100.9	96.1	143	28	101.2	94.5	150
WH.....	103.5	98.2	168	11	101.7	96.0	144	13	101.7	96.3	148
Ave.....	103.3	98.4	178	18	101.4	96.3	150	21	102.0	96.3	160

It is also important to know how long the state of acclimatization to heat lasts after exposures are discontinued. Three of the five subjects in this study were only slightly less efficient in the standard hot room experiments 2 to 3 weeks after stopping the exposures than at the end of acclimatization. This can be seen from the heart rates and body temperatures of the men (table 4). It is shown graphically for subjects WH and SR in figure 2. It should be noted that these two men performed their $4\frac{1}{2}$ hours' walks in the hot room 3 and 5 weeks respectively after the period of acclimatization was over. Subjects FC and HB, however, in repeating their walks 2 and 3 weeks respectively after stopping the exposures to heat had lost much of their acclimatization (table 4). This is probably related to the

fact that these two men had greater difficulty acclimatizing in the first place and never reached as complete a state of acclimatization as the other three subjects.

DISCUSSION. The most striking results of these experiments were: 1, the rapidity with which acclimatization occurred (about a week); 2, the shortness (1 to $1\frac{1}{2}$ hrs.) of the daily periods of work in the heat needed to produce acclimatization; 3, the retention of acclimatization by some of the subjects for periods of 3 weeks or more after exposures to heat ceased, and 4, the completeness of the acclimatization as evidenced by the fact that after acclimatization the men could maintain heat equilibrium in the standard grade of work about as well in the heated room as in the same work in a cool room.

These observations are obviously of great practical importance to industry and are even more important where rapid changes of climate by active men are necessary. Men who are already in good physical condition can be expected to work effectively within a few days after they start work in a hot climate; by a few relatively short daily exposures to work in artificially heated rooms they can be prepared for working immediately after they arrive in a hot climate. This process of acclimatization is not a substitute for the prolonged period of training necessary to produce good physical condition, but it is essential before men already in good physical condition can work effectively in the heat.

Additional experiments are needed to determine whether even more rapid acclimatization might be achieved by shorter, harder, more frequent work periods in the heat or by more prolonged daily periods of work.

The mechanisms involved in the improvement in performance of these men as they became acclimatized to the heat are not entirely clear from our data. The marked changes in heart rate and skin temperature of the men during acclimatization indicate that circulatory adjustments played an important part in the adaptation. From evidence cited above, these circulatory changes probably involved changes in peripheral circulation as well as in blood volume and tissue fluid volume. Since the men's skin temperatures during work in the heat were higher before than after acclimatization it is obvious that heat exchange by radiation was more favorable to the men before than after acclimatization. In the four men who showed decreases in their metabolic requirements for the standard work the average difference between the heat production during work before and after acclimatization was a decrease of 54 Calories per hour. This approximately equaled the average decrease of 51 Calories per hour in the rate of accumulation of stored body heat during work which occurred as a result of acclimatization. Since there was not a training effect the decrease in energy requirement for the work in the heat must be considered as a result of acclimatization as well as a contributor to it. The high rates of metabolism of these men during work in the early exposures to heat were not due to lack of training but apparently were associated with the high environmental temperature and the men's high body temperatures. This is evidenced by the fact that at the time of the first exposure to the heat all of the men were in good physical condition and were experienced in walking on the treadmill. HB had less experience on the treadmill than the others and yet he showed no training effect in his metabolic

requirement for the standard work—479 Cal./hr. in the cool room before exposure to heat, compared with 485 Cal./hr. after acclimatization to heat. These values are practically the same as similar determinations on him in the heat after acclimatization and significantly lower than in his initial exposure to heat (table 2).

One factor which contributed to the acclimatization of FC was his increased rate of sweating in the standard work experiments. Only 40 per cent of his increase in sweat secretion during acclimatization had to evaporate to account for his decrease in accumulation of body heat during work. It should be pointed out that in these experiments a considerable and unknown fraction of the sweat dripped off of the men and some accumulated in the clothing. The other subjects continued throughout acclimatization to sweat at about the same rate as in the initial experiments. The reason that these men did not increase their rates of sweating in the standard walks as did FC is that they showed greater decreases in body temperature during the walks as acclimatization proceeded (table 4). In their cases the work no longer elicited maximal rates of sweating (table 3).

The difference between FC and the other subjects in the manner and completeness of acclimatization to the heat may have been due in part to the fact that, although he was in good physical condition, he is a large, stocky man. He had 43 kgm. of body weight per square meter of body surface, as compared with 36 to 37 kgm. for each of the other four subjects. Since heat production in walking is proportional to body weight and heat dissipation depends largely on surface area, it is obvious that FC was handicapped in his heat regulation as compared with the other men (Robinson, 1942). In relation to surface area, FC sweated almost as much as the other subjects, whereas in relation to body weight, he sweated distinctly less than the others even after the gain he made during acclimatization. It is interesting that, although the rapid improvement in temperature regulation and comfort during the first few days of acclimatization may be accompanied by either a decrease in heat production or an increase in sweating or both, the continued slower improvement took place in these subjects without a further change in either of these processes. Therefore, the improvement in temperature regulation cannot be entirely dependent upon them.

SUMMARY

During the winter, experiments were carried out in which men walked on a motor driven treadmill from 1 to $1\frac{1}{2}$ hours a day in a room where desert conditions were simulated. When the men first began to take the walks the work was severe enough and sufficiently long to bring on symptoms of heat exhaustion.

The comfort and ease with which the men repeated the same walks which originally exhausted them increased rapidly during about 7 days and thereafter more slowly up to 23 days.

The heart rates of the men during the latter part of the walks declined from an average of 178 in the beginning to 155 on the seventh day.

The average skin temperature and rectal temperature of the men at the end of the work experiments declined from 98.4 to 96.5 F. and from 103.4 to 101.7 F. respectively during the same period.

This rapid improvement in temperature regulation during the first 7 days amounted to about 80 per cent of the entire improvement in 23 days. It was accompanied by an increase in the rate of sweating in one man and decreases in metabolic rate during work in the others. The slow improvement in temperature regulation occurring after the seventh day was not accompanied by continued lowering of metabolic rate nor by increase of sweating during the experiments. However, the capacity for sweating in harder work than the standard experiments did increase.

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CONSECUTIVE CHANGES IN CUTANEOUS BLOOD FLOW, TEMPERATURE, METABOLISM AND HEMATOCRIT READINGS DURING PROLONGED ANESTHESIA WITH MORPHINE AND BARBITAL¹

HAROLD D. GREEN, NEIL D. NICKERSON, ROBERT N. LEWIS AND
BERNARD L. BROFMAN

*From the Department of Physiology, School of Medicine, Western Reserve University,
Cleveland, O.*

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In the course of studies under prolonged anesthesia of the peripheral circulatory reactions during hemorrhage and shock, decreased cutaneous blood flow, rise in rectal temperature and oxygen consumption and fluctuations of hematocrit readings were observed during the first six hours after induction of the anesthesia which complicated the interpretation of the changes in peripheral circulation and metabolism occurring in the development of the shock state. These observations necessitated a study of the serial changes following the anesthesia alone, the results of which are presented in this paper.

METHODS. The effects of the various anesthetics were studied in 47 dogs weighing 7 to 20 kgm. The dogs were either unoperated or had inserted tracheal, arterial and venous cannulas. The following anesthetic combinations were given: 1, morphine sulphate and chloralose; 2, morphine and sodium pentobarbital; 3, morphine and sodium barbital; 4, sodium pentobarbital alone; 5, sodium barbital alone, or 6, morphine alone. In the following pages, where morphine and chloralose or a barbiturate are referred to, the morphine will be called the primary and the chloralose or barbiturate the secondary anesthesia. In all experiments the primary anesthetic dose of morphine was 2.0 to 4.4 mgm/kgm. The solutions administered were the standard preparations used in this laboratory for anesthesia. The morphine was given subcutaneously in the form of a 2 per cent solution in sterile distilled water. The sodium barbital was given as a 10 per cent solution prepared from "barbital sodium Merck, U.S.P.," the sodium pentobarbital was given as a 5 per cent solution prepared from "sodium pentobarbital powder, U.S.P.XI," purchased from Premo Pharmaceutical Laboratories, Inc.; and the chloralose was given as a 0.5 per cent solution. The solutions were freshly prepared and were dissolved either in sterile distilled water or in pyrogen-free water and administered either intravenously or intraperitoneally. Except for a slower action in the latter no significant difference was noted in either the dose required or the effects produced by the alternate routes of administration.

Subcutaneous temperatures were recorded in degrees centigrade with iron-constantin needle thermocouples inserted subcutaneously, and the body temperature from an iron-constantin couple inserted 10-15 cm. into the rectum, by

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means of a Leeds and Northrup 6 point Micromax recorder. The couples were automatically connected in turn to the recorder. The temperature of any one couple was recorded once every six minutes. The instrument is stated to have an accuracy of $\pm 0.3^{\circ}\text{C}$, but by keeping it carefully adjusted we were able to maintain an accuracy of the order of $\pm 0.2^{\circ}\text{C}$. Subcutaneous temperatures were found to be about 1° higher than surface temperatures but parallel to them, and less subject to error due to the fluctuating contact of the couple with the skin. The hair was clipped over the region of insertion of the thermocouples. In most of the experiments the animals were unheated and the rectal temperature was allowed to vary at will. In a few experiments, however, the rectal temperature was kept constant by varying the heating current supplied to two lamps placed under the animal board. Dark blinds were used at the windows and all drafts were avoided in order to maintain as even an environmental temperature as possible. The temperature of the air in the vicinity of the dogs was recorded with one of the needle thermocouples. Hematocrit readings were made of arterial blood with Wintrobe tubes. The results are expressed as cell volume per cent of total volume. One *point* is used as meaning 1 cell volume per cent. Oxygen consumption was measured with a recorder of the Sanborn blower type connected to a tracheal cannula. Blood pressure and heart rate were recorded by suitable optical manometers (1) or with a mercury manometer.

RESULTS. I. Rectal temperatures in unanesthetized dogs. Rectal temperatures, measured in 21 dogs immediately upon bringing them into the experimental laboratory from the animal house, ranged from 37.5° to 39.7° , with an average of 38.5° . The temperature ranged slightly higher during the summer than in the winter. These temperatures correspond well with those recently reported by Federov and Shur (2) and by Friedman and Bennett (3). Of 6 trained, unanesthetized dogs, 4 maintained their initial rectal temperature for the 1 to 4 hrs. during which they were kept lying quietly, but in 2 the rectal temperature dropped approximately 0.5° during the first 0.5 hr. before becoming stable at 38.8° and 39.0° . As shown in the first part of figure 2 this decline in rectal temperature was associated with a prominent rise in the subcutaneous temperature of the paws.

II. Subcutaneous and rectal temperatures. a. Morphine plus chloralose, sodium barbital or sodium pentobarbital. Two dogs were given morphine sulphate followed in 3 hrs. and in 1 hr. by 50 and 73 mgm/kgm. respectively of chloralose; 6 were given morphine followed in 0.25 to 1.25 hrs. by 160 to 200 mgm/kgm. of sodium barbital and 7 received morphine followed in 0.75 to 2.5 hrs. by 25 to 30 mgm/kgm. of sodium pentobarbital. The results were essentially the same with all three anesthetic combinations. Those from a typical experiment are illustrated in figure 1. In this experiment the rectal temperature had fallen to 35°C by the time the temperature recorder was connected. It reached a minimum of 32° five hours after giving the secondary anesthetic, after which it rose and stabilized at 38.6°C . At this time the animal was still deeply anesthetized. When recording began the temperatures of the skin over the thorax,

over the knee and on the dorsum of the hind paw were within 2° to 3° of the rectal temperature. Throughout the experiment the former two maintained about the same relation to the rectal temperature but about 2.7 hrs. after giving the barbital the temperature of the skin on the dorsum of the paw began to decline at the rate of 1.5° per hour. Prominent shivering such as was noted in this experiment was seen during the rise of the rectal temperature in 5 of these experiments.

In 10 of this group of 15 dogs, receiving morphine plus chloralose or a barbiturate, lowering of the subcutaneous temperature of the paws by 4.7° to 11.5° was noted within 0.75 to 6 hrs. after giving the secondary anesthetic. The rectal temperatures ranged from 34° to 38.8° when the drop in subcutaneous temperature occurred. In all but 1 of these dogs the rectal temperatures started to rise about the same time as, or shortly after, the subcutaneous temperature dropped. Within 3 to 11 hrs. after giving the secondary anesthetic, the rectal temperature of all 15 of the dogs had risen to 38.6° – 40.5° . In 4 of these dogs the rectal temperature continued to rise for 10 to 40 hrs. reaching levels of 39.5° to 42.6° . In 3 of the animals the subcutaneous temperature of the paws rose abruptly after the rectal temperature reached 39° to 40° .

The somewhat diphasic character of the above responses suggests that the morphine and barbiturate may have partially antagonistic reactions. The diphasic nature is more accentuated in those experiments in which recording was begun prior to the administration of morphine and in which the interval separating the primary and secondary anesthetic injections was increased. The results in a typical experiment of this type are reproduced in figure 2. Within six minutes after giving morphine the rectal and thoracic subcutaneous temperatures began to decline and the subcutaneous temperatures of the paws began to rise. The latter reached a maximum at 0.3 hr. and then dropped at a rate of 2° to 3° per hour. Again within 6 minutes after the intravenous injection of chloralose the subcutaneous temperatures of the paws suddenly rose, associated with a further fall of rectal temperature and of the subcutaneous temperatures of the ear and thorax. When the rectal temperature reached 36° the subcutaneous temperature of the paws began to decline at a rate of 5° to 6° per hour, and within 1.3 hrs. the rectal temperature began to rise. No shivering was seen in this experiment. The subcutaneous temperature of the fore-paw reached a minimum of 26° within 2.5 hrs. and the rectal temperature rose to a maximum of 38.9° within about 5 hrs. after administration of the secondary anesthetic. The subcutaneous temperature over the thorax remained within 1.5° to 2.5° of the rectal temperature. The subcutaneous temperature of the ear declined during the rise in rectal temperature. This was twice replaced by intervals of elevated temperature when small intravenous injections of sodium barbital were given.

II-b. *Sodium barbital and sodium pentobarbital alone.* Four dogs were anesthetized with 200 mgm/kgm. of sodium barbital, and two dogs with 25 and 35 mgm/kgm. of sodium pentobarbital alone. In all 6 of the animals anesthetized with a barbiturate the initial drop in rectal temperature was small

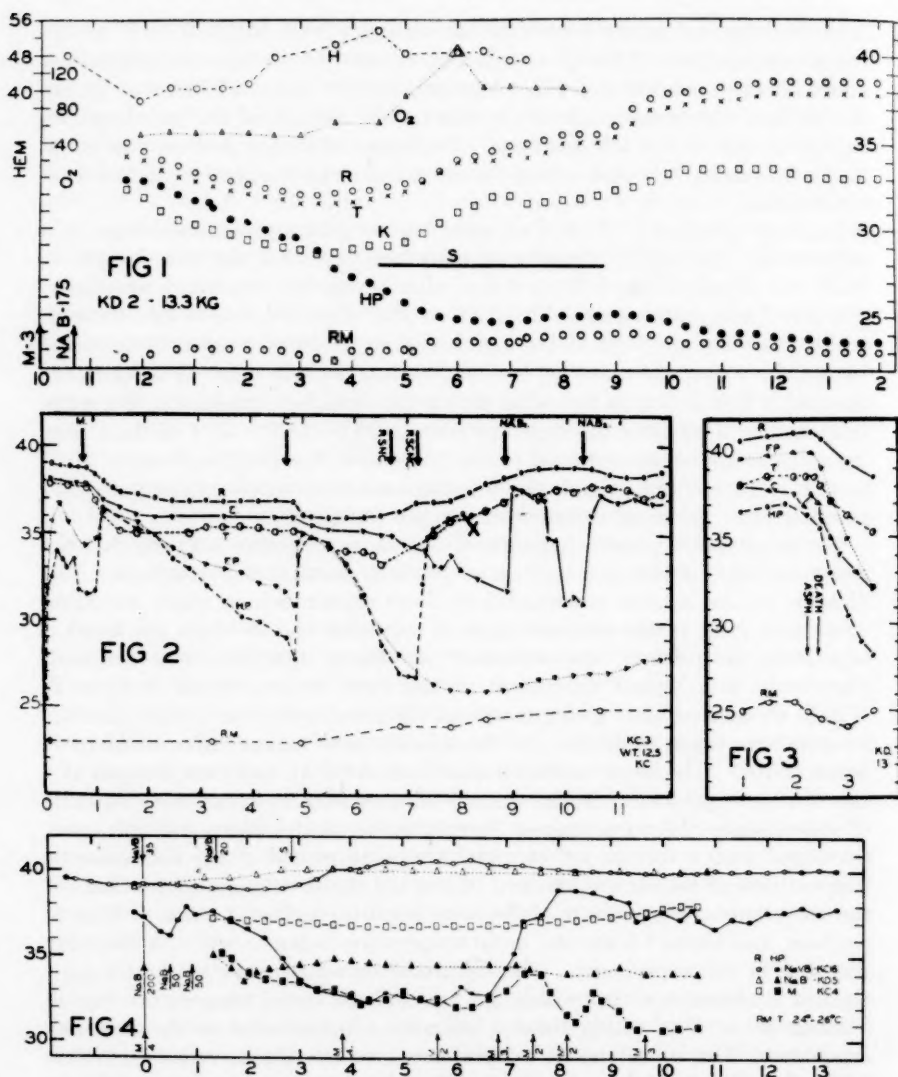


Fig. 1. Consecutive changes in rectal and subcutaneous temperatures, hematoctrit readings and oxygen consumption in a dog anesthetized with morphine and sodium barbital. *H*—hematoctrit reading, scale at left (*HEM*) = cell volume per cent of total volume; *O₂*—oxygen consumption, scale at left = *O₂* consumption in ml/min; *R*—rectal temperature, *T*—subcutaneous temperature over thorax, *K*—subcutaneous temperature in region of knee, *HP*—subcutaneous temperature on dorsum of hind foot, *RM*—air temperature near animal, scale for temperature at right in degrees centigrade; *S*—duration of observed shivering; *M-3*—subcutaneous injection of 3 mgm/kgm. of morphine; *NA-B-175*—intravenous injection of 175 mgm/kgm. of sodium barbital; abscissal scale—time in hours. Note all subsequent charts are drawn to the same scale as this chart.

(0.5° to 1.5°C) and in from 6 to 12 hrs. the rectal temperature had risen to 39.0 to 40.5°C and to 40.0° to 41.7°C after 15 to 28 hrs. Despite the smaller initial drop in rectal temperature there was in many of the barbiturate experiments a decline in the subcutaneous temperature preceding the rise of rectal temperature. The degree of lowering of the rectal temperature was usually less prominent and subsequent rise more marked than when morphine had been given preceding the barbiturate. Either alone or with morphine, sodium pentobarbital caused an earlier and more prominent rise of rectal temperature than sodium barbital. The results in two typical experiments are reproduced in figure 4.

II-c. *Morphine alone.* All but 1 of the 12 dogs, in which morphine alone was used, were anesthetized sufficiently to allow surgical procedures. This required from 4 to 20 mgm. per kgm. initially and a maintenance dose of 0.7 to 4.2 mgm. per kgm. per hour. In 8 of the 10 dogs studied at room temperatures of 23° to 26° the rectal temperature fell to between 34° and 37° within 1.5 to 4 hrs. Four showed no subsequent rise or decline and 4 showed a rise of less than 0.5°. In these dogs the subcutaneous temperature of the hind paws declined 1.2° to 10.2° within 1 hr. and 20 min. to 5 hrs. This drop in subcutaneous temperature occurred usually when the rectal temperature was around 35.9° to 37.8°, although in two instances it occurred at higher rectal temperatures. One animal was heated and in one the rectal temperature rose spontaneously. In

Fig. 2. Rectal and subcutaneous temperatures in a dog anesthetized with morphine and chloralosane, with prolonged interval between the two injections. Effect of cutting vasomotor nerves on subcutaneous temperature. *E*—subcutaneous temperature of outer surface of ear; *FP*—subcutaneous temperature of dorsum of fore-paw; *HP*—subcutaneous temperature of dorsum of right hind-paw; *X*—cessation of registration of subcutaneous temperature of hind paw; *M*—subcutaneous injection of 3.2 mgm/kgm. of morphine; *C*—intravenous injection of 50 mgm/kgm. of chloralosane; *RSNC*—section of right saphenous nerve; *RSCNC*—section of right sciatic nerve; *NAB₁* and *NAB₂*—intravenous injections of 80 mgm/kgm. of sodium barbital. Temperature scale at left in degrees centigrade; other lettering and scales as in figure 1.

Fig. 3. Rate of decline of subcutaneous temperature upon cessation of circulation. *C*—subcutaneous temperature of skin over calf; *ASPH*—asphyxiation; *DEATH*—cessation of cardiac and respiratory action as result of the asphyxiation. Other lettering and scales as in figures 1 and 2.

Fig. 4. Superimposed curves of rectal temperatures and subcutaneous temperatures of hind paw in dogs anesthetized with sodium pentobarbital, with sodium barbital and with morphine alone. Open symbols—rectal temperatures; solid symbols—subcutaneous temperatures of dorsum of hind paw. Circles—temperatures from dog anesthetized with sodium pentobarbital, intravenous injections of 35 and 20 mgm/kgm. indicated by upper row of arrows, *S*—onset of shivering in this animal, weight—18 kgm. Note in the original this record extended for another 7 hrs. with a rise of rectal temperature to 41.5°C 3 hrs. after the end of the reproduced segment of the record followed by a gradual decline to normal. The dog was returned to the animal cage at the end of this time and remained in good health. Triangles—temperatures from dog anesthetized with sodium barbital, intravenous injections of 200, 50 and 50 mgm/kgm. at times indicated by middle row of arrows, weight 18.5 kgm. Squares—temperatures from dog anesthetized with morphine, injections of 4, 4, 2, 3, 2, 2, and 3 mgm/kgm. at times indicated by bottom row of arrows, weight 10 kgm. Room temperature for all three experiments ranged between 24 and 26°C. Scales as in figures 1 and 2.

both animals when the rectal temperature reached 39° the subcutaneous temperature of the paws suddenly rose several degrees. In the 2 dogs studied at room temperature of 28° to 30° the rectal temperature remained within 0.5° of the pre-anesthetic level and the subcutaneous temperature remained within 3° of rectal temperature throughout the experiment. Shivering was not noted in any of the experiments with morphine alone. The results in one experiment are reproduced in figure 4.

III. *Effects of environmental temperature.* Room temperatures ranged in the different experiments from 21.5° to 30° , but most of the experiments were performed at room temperatures of 24° to 26°C . The variation in any one experiment was rarely more than 1.5° to 2.0° . Changes of subcutaneous and rectal temperature were observed in some animals at all environmental temperatures. However, with room temperatures of 28° to 29°C or higher it was observed that there was rarely much decline in subcutaneous temperature with or preceding the rise of rectal temperature; that the rectal temperature of dogs anesthetized with morphine did not decline appreciably; and that the rectal temperature of dogs anesthetized with a barbiturate rose higher than it did with dogs at lower room temperatures.

IV. *Evaluation of subcutaneous temperature changes in terms of blood flow.* Sudden maximal increase in blood flow was induced by cutting the sciatic nerve in 3 animals anesthetized with morphine alone and in 3 animals anesthetized with morphine and chloralosane during periods of lowered and of moderately elevated subcutaneous temperature. Prior cutting of the saphenous nerve had no effect, but in each instance after section of the sciatic nerve the subcutaneous temperature of the hind paw began to rise within six minutes and within 20 to 30 min. was within 1° to 2° of the rectal temperature. An example of this reaction is seen in figure 2.

The effect of sudden cessation of blood flow was studied by recording temperatures for 0.5 to 2 hrs. after death in 15 animals. When at death there was a difference of 10° to 15° between the recorded temperature and the room temperature the following initial rates of decline were observed: rectal temperature 1.0° to 2.0° per hour, thoracic subcutaneous temperature 2.0° to 3.0° per hour, subcutaneous temperature over the mid-region of the extremities— 2° to 4° per hour and over the paws 4° to 8° per hour. These data are illustrated in figure 3. When the thorax was opened, allowing the thoracic contents to drop away from the chest wall, the rate of decline of the thoracic subcutaneous temperature approached that of the paws.

Discussion. When the temperatures of the room and of the subcutaneous tissues are remaining constant an equilibrium exists between the rate of elimination of heat from the skin and the rate at which heat is being brought to the skin by the circulation and by direct conduction from the deeper structures. If in a given region heat is supplied almost solely by the blood and if the rate of circulation is low the equilibrium temperature is at or only slightly above room temperature, whereas if the rate of circulation is rapid the subcutaneous temperature will approach the internal body temperature as reflected by the rectal temperature.

Temperatures in between these two extremes will then, as suggested by Burton (4), indicate intermediate rates of flow. This arrangement appears to apply, however, only to areas of the skin, such as the ear and paws, which lie over regions of low heat content or ability to generate heat, and which are dependent almost entirely upon heat conducted to them by the blood stream. As indicated above, apparently the skin in the proximal portions of the extremities over large muscle masses, and especially the skin over the thorax and abdomen, receive heat by direct conduction from the deeper structures at such a rapid rate that the equilibrium temperature is determined by this mode of conduction rather than by the influence of any changes in the rate of heat conduction by the blood stream. As a result, as shown in figures 1 and 2, despite evidence of marked reduction in blood flow in the skin over the paws and ear which presumably would be accompanied by similar changes in the remainder of the skin, the subcutaneous temperature of the latter regions of the skin remains quite close to the rectal temperature. A corollary of this observation is the conclusion that, while marked generalized cutaneous vasoconstriction would reduce the heat loss from the skin over the extremities and similar regions, it would have only a minimal effect on the heat loss from the skin over the trunk and large muscle masses.

The interpretation of changes of blood flow by means of the recorder used in these experiments is dependent upon having a sufficient difference of temperature between the room temperature and the rectal temperature that small changes in blood flow can be detected readily. As a practical rule we have found that a minimum of about 15° is desirable for this purpose. The onset of a change of blood flow is probably accompanied by beginning change of subcutaneous temperature with a lag of only 30 sec. to a minute (4). However, because of the construction of our recorder the apparent latency in our records may be anywhere up to six minutes. Interpretation of the degree of change of blood flow after the assumption of a new rate of flow can be made only in a roughly qualitative manner until the subcutaneous temperature has again come to equilibrium. During the period of changing subcutaneous temperature an estimate of the new rate of blood flow can be made only by comparing the *rate of change* of subcutaneous temperature with the rate of change which normally occurs after maximal increase of flow such as is induced by section of the vasomotor nerve (the sciatic for the hind paw) or maximal reduction of flow as by sudden death. On the basis of such comparisons we conclude that in many instances maximal increase of flow is induced by the administration of either morphine or barbitol and that when a reduction of blood flow accompanies a rise of rectal temperature it is likewise very close to maximal.

The initial increase in cutaneous blood flow and the resulting increased heat loss plus a reduction of the spontaneous muscular activity was probably responsible for the initial drop in rectal temperature. Their occurrence may have been due to a depression of that center in the hypothalamic region which prevents fall of body temperature (Ranson, Fisher and Ingram (5)). The barbiturates and morphine seemed to potentiate each other slightly in causing

this initial effect. The subsequent decrease of cutaneous blood flow, the shivering and the rise of body temperature to and frequently well above normal with the barbiturates may have been due to a differential wearing off of the anesthetic depression of that part of the temperature-regulating center which prevents a fall of body temperature, while the depression of the center which prevents an overshooting of the body temperature (5) was still present. On the other hand, it could be equally well argued that the febrile like reaction, which was similar to that described by Du Bois (6) during the chill stage in man, was due to the presence of pyrogenic substances present in the various powdered preparations used. However, studies were carried out on 6 additional dogs using Abbott's Veterinary Nembutal (sodium pentobarbital) solution which is specially prepared for intravenous use, and the results were identical with those described above. Regardless of whether the sequence of temperature changes is due to the effect of the barbiturate per se or to the presence of an unrecognized pyrogen contaminant, allowance must be made for these changes when studying the peripheral circulation in animals anesthetized with these preparations.

V. *Oxygen consumption.* Throughout periods of anesthesia with morphine alone the oxygen consumption showed minor fluctuations but tended to remain relatively constant or to parallel the rectal temperature. However, as seen in figure 1, with morphine plus one of the barbiturates and with barbital alone there was usually a rapid rise in oxygen consumption to 125 to 200 per cent of the initial value during the period of vasoconstriction and rising rectal temperature, even in the absence of visible shivering. The O_2 consumption often dropped slightly when the rectal temperature became stabilized but remained elevated above the level present immediately after the animal was anesthetized.

Discussion. Using the Meeh-Rubner equation (6) for computing the surface area, $S = \frac{11.2 \times W^{2/3}}{100}$, S = surface area in meters,² W = weight in kgm., and assuming 1 liter of oxygen has a calorific value of 4.8, the metabolic rates under morphine anesthesia ranged from 33.2 to 51.8 Cal/M²/hour; under morphine and sodium barbital the metabolic rates ranged from 23.7 to 34.6; and under sodium barbital alone they ranged from 38.9 to 62.3. These were taken either during the initial depression of rectal temperature or after the rectal temperature had stabilized at a subsequent higher level. They show a rather wide scatter. When, however, the metabolic rates were plotted against the rectal temperatures the spread at any given temperature was much smaller, ranging at 38.5°C between 35 and 51 Cal/M²/hour. Comparison of pairs of readings from the same animal indicated that on the average the metabolic rate increased about 10 per cent per degree centigrade increase of rectal temperature. The discrepancy in the case of those that did not change characteristically with rectal temperature was probably due to increases in muscle tone which were not recognized as shivering. Metabolic rates for trained animals reported in the literature range from 24.6 to 39.2, using the same formula for calculating surface area (7-11). It appears that under morphine alone or morphine and barbital the metabolic rates are within the upper range of those found in unanesthetized animals, while those with sodium barbital alone are higher.

The initial increase in cutaneous blood flow and slight drop in rectal temperature during a barbiturate anesthesia are in agreement with the observations described by Richter and Oughterson (12) by Deuel, Chambers and Milhorat (13) and by Hemingway (14). The subsequent elevation of the rectal temperature to and often above normal has apparently not been previously observed in the dog but was seen in a patient by Edmonson (15). Diminished oxygen consumption has been observed after anesthetization with barbital by Anderson, Chen and Leake (16), with sodium amytal by Dameshek, Myerson and Loman (17) and Shapiro (18), and after delvinal sodium by Peoples and Carmichael (18). However, none of these investigators followed the metabolism for longer than 0.25 to 3 hrs. Increased oxygen consumption has been reported in cats under chloralose anesthesia by Griffiths, Emery and Lockwood (20). The complete sequence of changes such as we have described has not, to our knowledge, been reported.

VI. *Hematocrit readings.* The initial pre-anesthetic hematocrit reading in 19 dogs ranged from 41.1 to 56.4 with an average of 49.6. Within 0.5 hr. after the injection of barbital or pentobarbital the hematocrit reading usually decreased 5 to 11 points below the initial reading taken prior to the injection. This drop coincided with the increase in cutaneous blood flow. The absence of a diminution in three experiments may have been due to the fact that the second sample was not taken soon enough after giving the secondary anesthetic. The hematocrit reading began to increase again within 0.5 to 1.5 hrs. that is, when the rectal temperature was beginning to rise and the subcutaneous temperature to drop. The reading usually became stabilized at 2 points below to 14 points above the initial reading within 2.5 to 5 hrs.; that is, at about the time the subcutaneous temperature reached a minimum; the average in 12 experiments was at 3 points above the initial reading. The serial changes in hematocrit values in several typical experiments are reproduced in figure 5. With morphine alone the hematocrit reading usually showed little change throughout the period of anesthesia. The results in four experiments are reproduced in figure 6. Since, during the stable period the hematocrit readings tended to fluctuate by as much as ± 2 points, it would appear that a change in hematocrit reading induced by an experimental procedure would have to be greater than 2 points in order to be significant.

Discussion. The initial hemodilution confirms the observations of Jarcho (21), but this author apparently did not follow his animals long enough to observe the subsequent hemoconcentration. The explanation of the hematocrit changes cannot be stated with certainty on the basis of our experimental results. However, the association of the hemodilution with the increased blood flow and the hemoconcentration with the reduction of blood flow in the skin suggest that they probably are due to changes in the volume of blood in reservoirs such as the skin and spleen which may hold proportionally more red cells than plasma in the dilated state, and proportionally more plasma in the constricted state (22, 23). The association of such reactions with differential changes in splenic inflow and outflow described by Grindley, Herrick and Mann (24) and in spleen volume found by Seeley, Essex and Mann (25), and the reduction or abolition of the

hemodilution after splenectomy reported by Searles (26) and Adolph and Gerbasi (27) suggest that the spleen may be the chief reservoir in dogs. This is also suggested by the fact that marked hemodilution occurred in animals in which decrease in cutaneous blood flow was prevented by keeping the rectal temperature above normal (see below), and also by the small effects of increase and decrease of cutaneous blood flow upon the hematocrit in animals anesthetized with morphine.

VII. *Effects of artificially maintaining the rectal temperature constant.* In 8 dogs, anesthetized with sodium barbital alone, an attempt was made to avoid

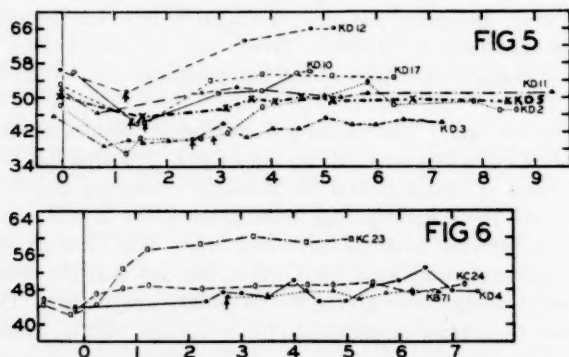


Fig. 5. Superimposed tracings of hematocrit readings from several experiments on dogs anesthetized with sodium barbital. *KD-2*—same experiment as reproduced in figure 1, morphine and sodium barbital, animal not heated; *KD-3*—morphine and sodium barbital, animal warmed sufficiently to prevent a drop in rectal temperature; *KD-5*—same experiment as in figure 4, sodium barbital only, not heated; *KD-10, 11, 12* and *17*—dogs anesthetized with sodium barbital alone and warmed sufficiently to maintain rectal temperature at 39°C. Small vertical arrows indicate moment when subcutaneous temperature of hind paws began to drop. This occurred despite the warming in experiments *KD12, KD10, KD17, KD2, KD3*. Points to the left of the solid vertical line are readings taken prior to administration of anesthetic. Ordinate scale—hematocrit reading in cell volume per cent of total volume. Abscissal scale—time in hours. This figure reproduced to same scale as figures 1 to 6.

Fig. 6. Superimposed tracings of hematocrit readings in four dogs anesthetized with repeated injections of morphine. For details see figure 5.

the fluctuations in temperature, metabolism and hematocrit by preventing the drop in rectal temperature during the first 4 to 6 hrs. by warming the animal board. When the animal was heated only enough to prevent a drop in rectal temperature below that observed at the time of anesthetization the reduction in cutaneous blood flow and augmentation of metabolism still occurred in 2-5 hrs. and the rectal temperature rose above normal. By maintaining the rectal temperature between 39° and 40°C, the fluctuations of rectal and cutaneous temperature and metabolism were minimized, but the characteristic decline and subsequent rise in hematocrit reading still persisted.

VIII. *Blood pressure and heart rate.* The mean arterial blood pressures and heart rates were not studied in all experiments. In those in which they were measured the initial mean blood pressures were highest with sodium barbital alone and with morphine and sodium barbital ranging in 20 experiments from 110 to 190 mm. Hg with an average of 148, and with chloralose, 140 and 160 mm. Hg. They were lowest with morphine alone (9 expts.) and with morphine and sodium pentobarbital (6 expts.) ranging from 96 to 170 mm. Hg with an average of 130 and 123 mm. Hg respectively. The lowest heart rates were seen with morphine alone and with morphine and chloralose ranging in 9 experiments from 50 to 90 beats per minutes, with an average of 64 beats per minute. The highest heart rates were found with sodium barbital alone, ranging in five experiments from 180 to 250, with an average of 214; and with morphine plus sodium barbital ranging in six experiments from 116 to 235 with an average of 174. In three experiments with morphine and sodium pentobarbital the heart rates were 100, 141 and 203 beats per minute. The mean blood pressure was practically unaffected by changes in blood flow in the skin, by shivering, or by a rise or decline of rectal temperature. The heart rate remained constant during the interval of declining cutaneous temperature, but not infrequently rose during and following the onset of shivering and rise of rectal temperature in animals anesthetized with one of the barbiturates.

Survival. In view of the interest at the present time in the survival of anesthetized animals after various experimental shock procedures, the following data are presented on the dogs used in this study. No significant difference was noted between the various anesthetic combinations. Twenty-nine of the 47 dogs were used for other experimental studies immediately after the conclusion of the period of temperature study. In so far as it is possible to judge, they remained in good condition up until they were sacrificed at the conclusion of the experimental period by bleeding or asphyxia. The total period of observation of these dogs was 8 to 26 hrs. (average—16 hrs.). Sixteen of the remaining 18 dogs were returned to the animal house in good condition and survived indefinitely; one died after 13 hrs.' anesthesia, apparently because of obstruction to the respiratory passage, and one was sacrificed at the end of a week because of the development of a respiratory infection. The average duration of the anesthesia in these 18 animals was: morphine and barbital or barbital alone—40 hrs.; morphine and sodium pentobarbital or sodium pentobarbital alone—11 hrs.; morphine alone $7\frac{1}{2}$ hrs.

SUMMARY

Roughly qualitative estimations of cutaneous blood flow are readily made in the skin of the paws and ears by recording the subcutaneous temperature with needle thermocouples connected with a Leeds and Northrup micromax recorder. If the subcutaneous temperature is remaining constant the blood flow may be estimated from the relationship of the subcutaneous temperature to the air and rectal temperatures. Subcutaneous temperatures approximating the former indicate minimal blood flow; those near the latter suggest maximal blood

flow. Immediately following a sudden change of blood flow the new rate of flow must be estimated by comparison of the ensuing rate of change of subcutaneous temperature with that observed to occur following the onset of a known change of blood flow. Estimation of the blood flow through the skin over the trunk or over large muscle masses in terms of the subcutaneous temperature is less satisfactory apparently because of the high rate of conduction of heat directly from the deeper structures.

The pre-anesthetic rectal temperature in our dogs ranged from 37.5 to 39.7 with an average of 38.5°C. Anesthesia with morphine alone caused an immediate and frequently maximal increase in cutaneous blood flow and a decline in rectal temperature to 34° to 37°C. These changes were often followed in 1 to 3 hrs. by a sharp reduction in cutaneous blood flow. The latter apparently was secondary to the temperature regulating reactions induced by the drop in rectal temperature. Sodium barbital, sodium pentobarbital and chloralose caused a similar initial increase in cutaneous blood flow and a drop of 0.5° to 1.5° in rectal temperature and a subsequent decrease of cutaneous blood flow; and in addition, induced an increase of oxygen consumption, often associated with shivering, and a rise of rectal temperature to 38.6° to 40.5° within 3 to 11 hrs. Anesthesia with morphine plus barbital accentuated the initial drop of rectal temperature.

Hematocrit readings showed little change with morphine alone, but with either morphine and a barbiturate or one of the barbiturates alone the hematocrit reading dropped 5 to 10 cell volumes per cent within a few minutes after anesthesia and then slowly returned to and often above normal during the period of reduction of cutaneous blood flow and rise of rectal temperature.

Changes in rectal temperature and cutaneous blood flow were minimized by maintaining the rectal temperature between 38° and 39° and were often abolished by elevating the rectal temperature to 39°-40° by warming the animal board. The heating had, however, no significant influence upon the initial decline and subsequent rise of the hematocrit reading in the dogs anesthetized with barbital. Both heart rate and mean arterial blood pressure were higher under sodium barbital anesthesia than under the other anesthetic combinations.

Because of the above sequence of events during the first hours after induction of anesthesia with any of these drugs we recommend that a control period of at least 3 to 4 or more hours elapse following anesthetization before proceeding with experimental studies, if hematocrit, oxygen consumption, rectal temperature, or cutaneous blood flow are to be studied. Such a control period is desirable even when the rectal temperature is being maintained constant by warming the animal.

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METABOLISM OF THE PERFUSED DOG'S BRAIN¹

CARROLL A. HANDLEY, H. MORROW SWEENEY,² QUINTEN SCHERMAN AND
ROBERT SEVERANCE

*From the Department of Physiology and Pharmacology, University of South Dakota School of
Medical Sciences, Vermillion*

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Values for the "in vivo" oxygen consumption of the brain found in the literature are usually regarded as rough approximations. These figures are based on indirect measurements of blood flow through the brain made by recording either the internal carotid blood flow with a stromuhr or by measuring the venous return from the brain. The arterio-venous oxygen differences were then used to calculate brain oxygen consumption. The existence of anastomotic branches (see fig. 1) between the internal and external carotid arteries indicates that internal carotid artery blood flow is not identical to cerebral blood flow in the dog; moreover, the external jugular veins of the dog carry blood from other structures of the head as well as the brain.

The gross discrepancy between the "in vivo" and "in vitro" values for brain oxygen consumption has been discussed in a recent review (1). Oxygen consumption figures reported for brain tissue slices or brei provide much useful information; but these figures can hardly be expected to approach the actual values for the intact living brain because of the damage to the tissue from the mincing or slicing, the anoxia incurred during the preparation and the artificiality of the medium in which the tissue is placed.

The present report is concerned with some aspects of brain metabolism obtained with a method of perfusing dog's brains in which the circulation was isolated from other tissues as far as could be ascertained by the injection of dyes.

METHODS. Blood for the perfusion was defibrinated as withdrawn from a large dog which was sacrificed. The lungs were removed to use for oxygenating the blood during the experiment, and the pulmonary artery was cannulated close to its origin. The return flow from the pulmonary veins was collected by tying a large cannula into the left auricular appendage and occluding the bicuspid valve by means of a ligature around the auricular ventricular groove. Saline solution was passed through the lung system to wash out the blood and the lungs were suspended in a large container that served as a moisture chamber. The pulmonary artery was connected to one unit of a double pump of the Dale-Schuster type (2). Blood at 37°C. was pumped through the lung system and passed into a reservoir under the moisture chamber. The blood in the reservoir was covered by a layer of mineral oil to reduce the rate of carbon dioxide loss. From the reservoir, the blood returned to the perfusion pump. The defibrinated

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² Captain, AC, Aero Medical Laboratory, Wright Field, Dayton, Ohio. On leave from the University while on active duty.

blood was circulated through the lungs for thirty minutes or more before beginning the head perfusion to remove vasoconstrictor substances (3).

A second dog was prepared for brain perfusion under ether anesthesia. The superior sagittal sinus was exposed by trephining the skull over the vessel and elongating the opening with rongeurs. The carotid arteries were then exposed and all muscles branches of the external carotid ligated, as indicated in figure 1. In order to expose and ligate the internal maxillary arteries it was necessary to approach these vessels through the mouth. Incisions were made just behind and slightly medial to the last molars. Separation of the muscles exposed the maxillary arteries, which were ligated central to their ophthalmic branches. In confirmation of Bouckaert and Heymans (4), we found that when the vertebral arteries are ligated the blood flow through the internal carotid arteries is

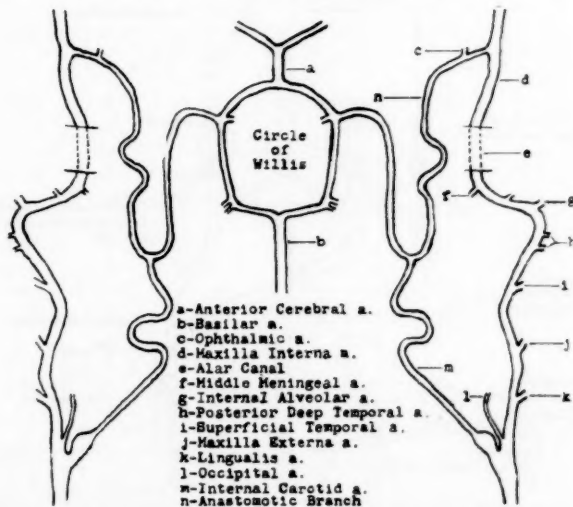


Fig. 1. Diagram of the blood supply of the brain in dogs. Drawn from preparations injected with colored latex.

not adequate to maintain the brain; the larger flow to the brain by way of the external carotid arteries and anastomotic branches (internal ophthalmic arteries) must be functional.

The head was connected to the perfusion pump by cannulating the external jugular veins and the common carotid arteries. This was done on one side at a time, so the circulation to the brain was at no time interrupted. As the head was being connected to the pump, the venous outflow was collected and defibrinated, since part of it came from the perfused dog and had not previously had the fibrin removed. The perfusion pressure was maintained at about the same level as the dog's carotid pressure. A heavy clamp was then tightened about the neck to cut off the blood flow to and from the brain through the vertebral arteries and plexus of veins on the spinal cord surface. Complete occlu-

sion of these arteries and veins proved to be difficult, but was finally accomplished by constructing a suitable clamp. Essentially, this device is a loop of steel cable that can be tightened to compress the vertebral column.

In the course of these experiments it was found that the carbon dioxide concentration in the perfusing blood was below physiological levels due to the necessary exposure of the blood to atmospheric air at two points in its passage through the circuit. Since the concentration of this gas in the blood plays a rôle in the maintenance of normal patency of vessels (5, 6, 7, 8), it was necessary to keep the gas within the normal range. The carbon dioxide tension of the perfused blood was controlled by using the apparatus shown in figure 2.

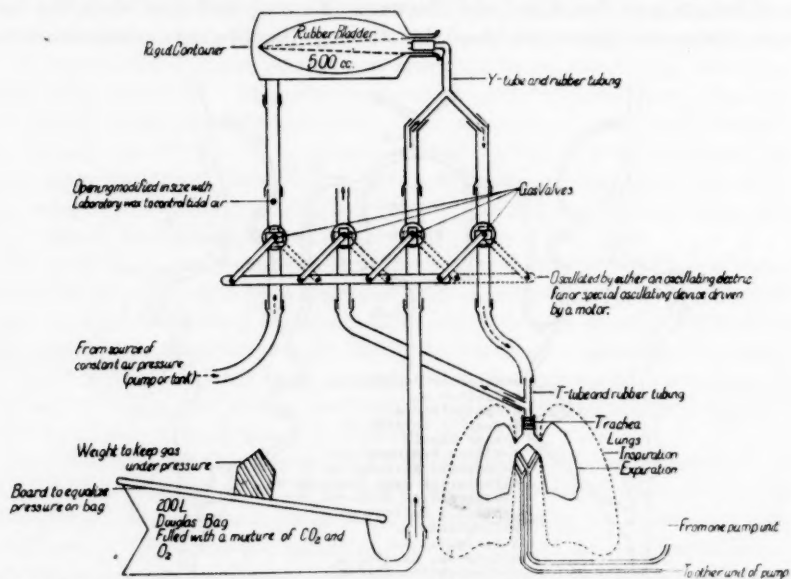


Fig. 2. Apparatus for ventilating the lungs. The solid lines represent the position of the mechanical parts and lungs when air is moving in the direction of the solid arrows. When the oscillating device moves the valves, as represented by the broken lines, the lungs are inflated by air moving as indicated by the broken line arrows.

The perfusion was allowed to proceed for twenty or thirty minutes to eliminate the ether. Blood samples were then simultaneously drawn from the superior sagittal sinus and carotid artery. At the same time the minute blood flow was measured by collecting the venous return from the head in a graduated cylinder. The blood to be used for gas analysis was immediately chilled in ice water. Blood used in the lactic acid and dextrose determinations was treated at once as the respective methods demand. Carbon dioxide and oxygen determinations were made according to the method of Van Slyke and Neill (9), blood sugar according to the method of Hagedorn and Jensen (10), and lactic acid by the method of Miller and Muntz (11) as modified by Barker and Summerson (12).

RESULTS. The results of these experiments, recorded in tables 1 and 2, show

TABLE 1

DOG NO.	TIME	SINUS AND ARTERY	CO ₂	A-S DIFFERENCE	O ₂	A-S DIFFERENCE	DEXTROSE	A-S DIFFERENCE	LACTIC ACID
			<i>vol. per cent</i>		<i>vol. per cent</i>		<i>mgm. per cent</i>		<i>mgm. per cent</i>
1	2:45	S	51.69	4.62	13.13	4.63	128	5	6.2
		A	47.07		17.76		133		6.8
	3:15	S	51.34	3.81	14.11	4.13	154	6	6.5
		A	47.53		18.24		160		6.1
	3:45	S	50.75	3.72	14.41	3.72	155	5	7.4
		A	47.03		18.13		160		6.9
2	2:30	S	30.81	5.29	6.07	5.76	87	11	7.7
		A	25.52		11.83		98		8.1
	3:00	S	34.53	4.91	9.88	5.37	107	7	9.0
		A	29.62		15.25		114		8.7
	3:30	S	32.59	5.43	10.56	5.55	83	8	8.2
		A	27.16		16.11		91		8.5
3	12:25	S	46.82	2.79	14.51	2.96	136	4	7.3
		A	44.03		17.47		140		6.8
	1:00	S	53.41	3.24	9.72	3.68	130	5	7.8
		A	50.17		13.40		135		7.3
4	1:30	S	35.68	5.64	13.27	5.37	150	6	9.3
		A	30.04		18.64		156		9.0
	1:50	S	42.49	4.22	12.00	4.57	140	5	8.4
		A	38.27		16.57		145		8.9
	2:30	S	47.31	4.66	12.77	4.56	144	5	8.7
		A	42.65		17.33		149		9.1
5	12:15	S	39.20	3.09	10.40	3.77	135	7	9.9
		A	36.11		14.17		142		9.6
	12:45	S	41.60	3.67	8.38	4.33	138	11	9.3
		A	37.93		12.71		149		8.8
6	1:30	S	38.86	3.06	3.92	3.16	94	6	7.6
		A	35.80		7.08		100		7.2
	2:00	S	39.97	2.93	5.57	3.17	61	6	7.3
		A	37.04		8.74		67		6.9
7	1:15	S	42.76	3.17	11.14	6.82	149	7	9.4
		A	39.59		17.96		156		8.7
	1:40	S	50.47	4.15	13.59	4.26			9.1
		A	46.32		17.85				9.5

that an average of 4.6 volumes percent of oxygen was removed by these brains as the blood passed through them. The figures for the minute oxygen consumption per 100 grams of brain tissue (table 2) show a fairly wide variation among the different experiments; although the cerebral oxygen consumption in any one dog appears more or less constant from time to time, under the conditions of the experiment. The average value for the seven experiments is 10.9 cc. per 100 grams of brain per minute, a value that is in fairly close agreement with results

TABLE 2

DOG NO.	WEIGHT	BLOOD FLOW		PERFUSION PRESSURE	MIN. CONSUMPTION PER 100 GRAMS BRAIN		QO ₂
		cc./min.	Per 100 grams Brain/min.		Oxygen	Dextrose	
	<i>kgm.</i>				<i>cc.</i>	<i>mgm.</i>	
1	13.6	160	245	150	11.0	11	6.6
		170	260	150	10.5	9	6.3
		170	250	155	9.7	11	5.8
2	17.3	176	240	140	13.6	15	8.1
		142	192	150	10.7	13	6.4
		140	188	155	10.9	15	6.7
3	19.4	224	340	118	10.2	13	6.1
		204	310	118	11.3	15	6.8
4	18.6	184	240	120	12.9	14	7.7
		212	265	130	12.6	13	7.5
		208	270	125	12.6	12	7.5
5	13.6	260	330	118	12.1	23	7.2
		178	230	160	9.9	20	5.9
6	11.8	240	310	120	9.8	18	5.9
		220	280	130	9.1	16	5.5
7	12.3	96	135	85	9.3	10	5.6
		160	220	120	9.5		5.8
Averages.....					10.9	14	6.8

obtained by less direct means (13, 14, 15). Calculations of the QO₂ values (cmm. oxygen consumed per mgm. of brain tissue per hr.) gives an average of 6.8.

During the passage of blood through the brain there was an average uptake of 7 mgm. per cent of dextrose. The average dextrose consumption per 100 grams of brain per minute was 14 mgm. The whole brain, in these experiments, used about 0.5 gram of dextrose per hour; and in order to keep the blood sugar level within normal limits, small amounts of sugar had to be added to the perfusing blood from time to time.

No evidence was obtained from these experiments to indicate lactic acid formation or utilization by the brain. The lactate content of the arterial and venous blood remained the same throughout an experiment.

DISCUSSION. The viability of the preparation could readily be ascertained by observing the eye reflexes. The pupillary and palpebral reflexes are more sensitive to oxygen lack than are certain of the vital centers, such as the cardiac, vasomotor and respiratory centers (16). Furthermore, the work of McFarland, Knehr and Behrens (17) indicates that ocular activity is a sensitive indicator of hypoxia of the brain. The blood supply to the eyeball was therefore left intact. Trial experiments demonstrated that the blood flow to the eye is too small, in comparison with the total cranial flow, to introduce a significant error in calculating the oxygen consumption figures for the brain.

An important question to be answered is whether the perfusion was limited to the brain tissue alone. At the conclusion of each experiment a dye was injected into the arterial blood flow to the brain. In no case did more than insig-

TABLE 3

DOG NO.	O ₂ CONSUMPTION OF INTACT DOG	O ₂ CONSUMPTION OF BRAIN	PER CENT OF TOTAL CON- SUMED BY THE BRAIN
	<i>cc. per minute</i>	<i>cc. per minute</i>	
1	101	7.4	7.3
2	128	10.2	8.0
3	144	9.5	6.6
4	138	11.1	8.1
5	103	8.5	8.2
6	87	7.3	8.4
7	91	6.7	7.4
Average.....			7.7

nificant amounts of dye appear in tissues other than brain substance and the eyeball. The dye appeared in the brain tissue down to the lower end of the medulla. The brain was sectioned at this point and removed and weighed.

The figures for blood flow cannot be regarded as comparable to cerebral blood flow in the normal animal. Although the mechanical pump delivered pulsatile flow and the perfusion pressure was maintained at approximately the carotid pressure of the normal dog, the small arterio-venous oxygen differences, when compared with values reported in the literature, indicate that the blood flows were greater than normal.

The blood gas values of sinus blood and the venous outflow from the head were the same, a further indication that no significant blood flow to tissues other than the brain occurred. Preliminary perfusion experiments in which all the muscular branches of the carotid arteries were not ligated gave an apparent oxygen consumption of 20.5 cc. per 100 grams of brain, a figure nearly double the value obtained after ligation of the extra-cranial arteries.

With the limitations in mind of determining the respiratory quotient of tissues

by arterio-venous blood gas differences (19), the results of these experiments are perhaps noteworthy in that they agree closely with earlier observations on the R.Q. of the brain. Lennox (19) found an average brain R.Q. of 0.95 in human subjects and Himwich and Nahum (15) reported a value of 1.0 for dogs. In the sixteen observations of these experiments, the average brain R.Q. is 0.94 (excluding the first observation of expt. 7 which is inexplicably low).

Table 3 shows a comparison of the basal oxygen consumption of the intact dog with that of the brain.

The Q_{O_2} values are calculated on the basis of the wet weight of the brain. They may be converted to dry weight figures by multiplying by the factor 5 (18).

SUMMARY

A method of isolating the circulation to the dog's brain and a perfusion technique are described. The average minute oxygen consumption per 100 grams of brain in seven experiments, using the above technique, was 10.9 cc. giving a Q_{O_2} of 6.8. The average brain R.Q. was 0.94. Observations on dextrose and lactic acid metabolism by the brain are also recorded. These experiments indicate that approximately 50 per cent of the oxygen supplied to the head region is used by the brain and that the brain accounts for about 8 per cent of the total oxygen consumption of the body at rest.

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THE EFFECTIVENESS OF PLASMA, GELATIN AND SALINE TRANSFUSIONS IN PREVENTING SHOCK INDUCED BY LEG MUSCLE TRAUMA AND TOURNIQUETS¹

W. KLEINBERG, J. W. REMINGTON,² W. J. EVERSOLE, R. R. OVERMAN AND
W. W. SWINGLE

*From the Section of Physiology, Biological Laboratory, Princeton University, Princeton,
New Jersey*

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In a study in which shock was produced in dogs by the release of tight leg tourniquets after a five hour period of constriction, it was observed that saline infusions and a single large plasma transfusion could prevent death in but a few of the cases (1). When, however, the same amount of plasma was divided into five equal doses, and given intermittently over the whole of the critical period, shock was prevented in all cases. These results led us to make a comparative study of the effectiveness of plasma, gelatin and saline in preventing shock following leg tourniquet release, and also that following leg muscle trauma. Both the single infusion and intermittent injections were employed for the administration of the plasma and plasma substitutes.

Gelatin has been used in the treatment of shock by several groups of investigators within recent years (2-9). Their reports indicate that its effectiveness in restoring a depleted plasma volume after massive hemorrhage or after burns is somewhat less than that of plasma, but greater than that of a salt or other crystalloidal solution. Since different lots of gelatin, even when prepared in identical fashion, may vary somewhat, care was taken to reduce to a minimum the number of different batches used. All experiments were done with gelatin from two different lots.

A. *Tourniquet shock.* As in the experiments published earlier (1), shock was produced in a series of dogs by the tight application, as high on the hips of both legs as possible, of a heavy walled rubber tubing. In the previous publication, through a typographical error, the diameter of this tubing was quoted as 120 mm. instead of the correct 12 mm. The tourniquets were released after a period of 5 hours. The symptoms shown by the control animals for this experiment were identical in all respects with those previously described (1). There was a marked swelling of the injured legs, intense hemoconcentration (from 38 per cent hematocrit to 71 per cent), and a plasma volume reduction of 49 per cent. The evidence seemed clear that the essential factor contributing to the initiation of the shock condition was the extreme local fluid loss into the injured legs.

A plasma transfusion of 25 cc. per kgm. body weight given either immediately after release of the constrictions, or later, ameliorated somewhat the extent of

¹ The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Princeton University.

² Upjohn Research Fellow.

the hemoconcentration, but failed to prevent shock in 9 of 12 dogs (1). Saline infusions were even less effective. When the same amount of plasma was divided into doses of 5 cc. per kgm. body weight each, and given at 0, 1, 2, 4 and 7 hours after tourniquet release, all signs of shock were prevented in all cases of a series of 7 dogs. The positive effect could not be correlated with hemoconcentration changes.

In the present experiments, 25 cc. per kgm. body weight of a 5 per cent gelatin solution, prepared in physiological salt solution with an oncotic pressure of 70 mm. Hg after sterilization³, was divided into 5 equal doses for intermittent transfusions into a series of 14 dogs. Thirteen of the transfused animals of this series showed no signs of shock at any time, and were eating full rations at the end of 24 hours from the time of tourniquet release. The single failure survived for 36 hours, maintaining a good arterial pressure throughout the interval, and then succumbed to a respiratory infection.

The other 13 dogs were kept ten days. The legs showed no development of gangrenous areas, nor were any ill effects of the gelatin observed. So far as

TABLE 1

Average blood pressure, pulse, and blood concentration changes in 14 dogs protected against shock following release of leg tourniquets by an intermittent transfusion of gelatin

TIME	BLOOD PRESSURE	PULSE PER MINUTE	HEMATOCRIT	HEMOGLOBIN	SERUM PROTEIN
	<i>mm. Hg</i>		<i>per cent</i>	<i>grams per cent</i>	<i>grams per cent</i>
Initial.....	104	130	40.2	14.3	5.72
Tourniquet release.....	102	133	50.8	17.7	6.59
4 hrs. after release.....	104	147	60.2	21.8	6.39
7 hrs. after release.....	99	149	60.5	21.2	6.27
24 hrs. after release.....	96	138	54.2	17.8	5.72

could be judged by blood pressure, hematocrit and hemoglobin changes, the gelatin was just as effective as plasma when given intermittently over the 7 hour period. In fact, the hemoconcentration tended to be less extreme than with plasma transfusions (table 1). Serum protein concentrations, measured as serum specific gravity by the falling drop apparatus, showed a slight fall in the gelatin infused animals, rather than the rise which follows plasma treatment (1). Since it had been shown previously that a single large transfusion of heparinized plasma was ineffective in this type of shock we did not attempt to test the gelatin by the single transfusion method.

B. Leg muscle trauma. The type of leg muscle trauma employed was similar to that first used by Kendrick, Essex and Helmholtz (10), Best and Solandt (11) and Cullen and associates (12), as later modified by Gregersen and co-workers (13). The animal is placed under ether anesthesia, and 400-800 blows delivered to all faces of the thigh muscles of each leg with a 200 gram rawhide mallet. The legs are numbed and severely bruised, but the skin is unbroken and no

³ We are indebted to Dr. John F. Norton and the Upjohn Company of Kalamazoo Michigan for generous supplies of specially prepared sterile gelatin.

bones are fractured. Trauma is continued until the mean arterial pressure in the femoral artery as determined by the needle puncture method (14), has been reduced to 60-70 mm. Hg. Ether is then discontinued, and the animal tied on its back on the animal board for 6 hours, after which, if still alive, he is returned to his cage. No food or water is given the animals which recover until after the 24th hour.

Of a control series of 37 dogs subjected to this form of muscle trauma, 33 died in shock and 4 recovered. The average life span was 5 hours (15). As has been

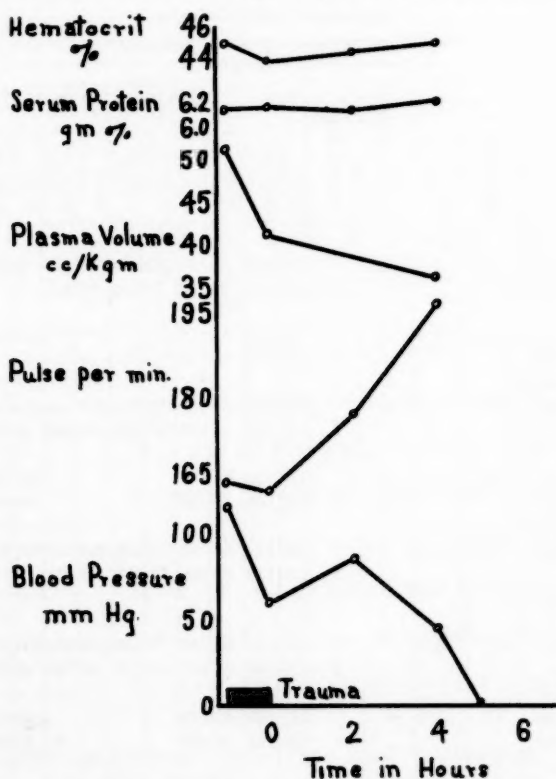


Fig. 1. Plasma volume changes after trauma

reported by others, the shock induced by this type of leg muscle trauma is not accompanied by consistent or significant changes in hematocrit, hemoglobin or serum protein. Blood changes shown by a representative animal are graphically presented in figure 1. The plasma volume, as measured by the dye T-1824 method (16), shows an average decline of 32 per cent before death. Detailed studies on the shock induced by leg muscle trauma will be reported later. We are concerned here solely with the effect of transfusions of plasma, gelatin and saline upon the prevention of shock induced by this procedure.

1. *Single transfusion of plasma.* In all plasma transfusion experiments, sterile heparinized plasma, obtained by rapid continuous bleeding of a large donor dog, was employed. Each of a group of 10 dogs, subjected to muscle traumatization, was given a single intravenous transfusion of 33 cc. per kgm. body weight. As with the controls, the blood pressure was reduced to below 70 mm. Hg before trauma was stopped. The pressure then showed a slow rise to 80-90 mm. Hg,

TABLE 2
Average blood pressure and pulse changes in traumatized dogs given plasma,
gelatin and saline infusion
Blood pressure given in mm. Hg

	NO. OF DOGS	BODY WEIGHT kgm.	NO. DOGS SURVIVED	NO. DOGS DIED	INITIAL		AFTER-TRAUMA		PRE-TRANSFUSION	POST-TRANSFUSION		6 HOURS		24 HOURS		SURVIVAL TIME hours
					Blood pressure	Pulse per minute	Blood pressure	Pulse per minute		Blood pressure	Pulse per minute	Blood pressure	Pulse per minute	Blood pressure	Pulse per minute	
Plasma, 33 cc./kgm., single transfusion	10	8.4	2	8	113 168	66 144	72 146	109 156	96 138	108 148						6
Plasma, 33 cc./kgm., intermittent transfusion	13	10.6	10	3	116 152	65 152						111 187	113 156			4
Gelatin, 33 cc./kgm., single transfusion	10	9.6	5	5	119 136	61 154	79 195	127 148	113 169	99 173						9
Gelatin, 33 cc./kgm., intermittent transfusion	10	9.6	6	4	121 159	58 158						107 167	107 158			10
1% salt solution, 33 cc./kgm., single transfusion	12	9.5	7	5	123 147	66 145	78 199	101 175	104 159	99 142						6
20% salt solution, 20 cc., single infusion	12	10.6	6	6	129 155	65 181	69 176	93 145	65 218							6
20% salt solution, 20 cc., intermittent infusion	12	10.6	6	6	122 165	62 145						105 154	120 130			8
15% salt solution, 30 cc., intermittent infusion	15	10.4	6	9	119 136	66 144						96 160	121 140			6

which level was maintained for a variable period. Transfusions were given only when the pressure level was again declining, and was below 85 mm. Hg (table 2). At least 1 hour was allowed from the time of the completion of the trauma. Of the 10 animals given the single plasma transfusion at this time, but 2 survived. Hematocrit and hemoglobin levels evidenced a moderate blood dilution, which was not rapidly lost (table 3). No correlation could be drawn between the

extent of this dilution and the symptoms of the animal. Serum protein concentrations were elevated.

2. *Intermittent transfusion of plasma.* An equivalent amount of plasma, i.e., 33 cc. per kgm. body weight, was divided into 5 equal doses, and given at 0, 1, 2, 4 and 7 hours after the trauma. Of 13 traumatized animals given this type of transfusion 10 showed no symptoms of shock and survived indefinitely, and 3 died in shock. Hemodilution changes were of the same general order as with the animals receiving the single plasma transfusion (table 3).

TABLE 3

Average blood concentration changes in traumatized dogs given plasma, gelatin and saline infusions

	NO. OF DOGS	INITIAL			½ HOUR AFTER TRANSFUSION			6 HOURS AFTER TRAUMA			24 HOURS AFTER TRAUMA		
		Hematocrit	Hemoglobin	Serum protein	Hematocrit	Hemoglobin	Serum protein	Hematocrit	Hemoglobin	Serum protein	Hematocrit	Hemoglobin	Serum protein
		per cent	gm. per cent	gm. per cent	per cent	gm. per cent	gm. per cent	per cent	gm. per cent	gm. per cent	per cent	gm. per cent	gm. per cent
Plasma, 33 cc./kgm., single transfusion.....	10	46.6	17.3	6.28	31.3	10.7	7.09	38.1	14.2	6.44			
Gelatin, 33 cc./kgm., single transfusion.....	10	47.0	16.5	5.93	28.4	10.3	4.96	36.4	13.0	4.90	42.3	14.9	6.09
Salt solution, 20%, 20 cc., single transfusion.....	12	47.5	17.3	6.12	44.5	16.6	5.86	43.9	17.2	6.03	39.5	13.8	5.11
Salt solution, 1%, 33 cc./kgm., single transfusion..	12	48.8	18.8	6.01	46.8	17.6	5.90	52.0	20.7	6.35	43.9	19.3	5.88
Plasma, 33 cc./kgm., intermittent transfusion.....	13	51.0	18.1	6.93	41.3	15.6	7.21	41.3	15.3	7.12	38.2	13.8	7.00
Gelatin, 33 cc./kgm., intermittent transfusion.....	10	49.2	17.0	6.67	41.8	15.0	6.13	42.1	15.4	5.71	50.7	14.9	6.21
Salt solution, 20%, 20 cc., intermittent transfusion..	12	46.3	16.1	6.02	41.4	14.4	5.78	39.0	14.6	5.80	36.0	13.2	5.47
Salt solution, 15%, 30 cc., intermittent transfusion..	15	49.6	17.7	6.50	47.5	17.4	6.40	44.4	16.4	6.25	40.4	13.8	5.62

The experiments demonstrate again that small intermittent transfusions distributed over a 7 hour interval are more efficacious in preventing shock, both after tourniquet release and after leg muscle trauma, than a single large transfusion.

3. *Single transfusion of gelatin.* A transfusion of 33 cc. per kgm. body weight of the 5 per cent gelatin solution was given at the time of the secondary blood pressure fall, and never less than 1 hour following the completion of the trauma. Of 10 dogs so transfused, 5 showed no signs of shock and survived, while 5 died in shock, with the survival span somewhat prolonged (table 2). Hemodilution was more marked than with the animals receiving plasma (table 3), but, again,

no correlation could be made between the extent of the dilution and the symptoms of the animal. Serum protein values showed a distinct decline rather than the rise which follows injection of plasma.

It should be noted that, in this experiment, the actual blood pressure level at the time the transfusion was begun (table 2) was lower in the animals which later succumbed than in those which recovered. In other words, the ineffectiveness of the gelatin infusion in 4 of the 10 animals might be correlated with the fact that the shock condition was more severe at the time the transfusion was started. Of the other 6, which showed blood pressure levels between 75 and 85 mm. Hg at the time the transfusion was started, shock was prevented in 5.

4. *Intermittent transfusion of gelatin.* Each of another series of 10 dogs was given the same total amounts of gelatin, 33 cc. per kgm. body weight, but divided into 5 equal doses, given at 0, 1, 2, 4 and 7 hours following conclusion of the trauma. Six of these animals exhibited no symptoms of shock, the other 4 died in shock, but the survival span was lengthened (table 2). The difference between the two modes of gelatin administration is not sufficiently clear cut in this experiment to permit a conclusion as to their relative merits.

5. *Single infusion of 1 per cent salt solution.* Since the gelatin was prepared in physiological saline, it seemed necessary to test the efficacy of saline alone in preventing shock. In the first of these experiments, 33 cc. per kgm. body weight of a 1 per cent salt solution was given as a single infusion at the time of the secondary blood pressure fall. Of a total of 12 dogs, 7 had arterial pressures between 75 and 85 mm. Hg at the time the infusion was begun and of these, 6 dogs lived indefinitely and 1 dog died after 12 hours. Of the other 5 animals which showed blood pressures below 75 mm. Hg when the infusion was begun, 1 dog recovered and 4 died in shock. Hence, for this whole series of 12 dogs, 7 recovered and 5 died in shock.

Hemodilution following the salt infusion was slight, and was not maintained (table 3). In fact, a tendency toward hemoconcentration followed within a few hours. Again, the survival of the animal could not be correlated with blood dilution.

6. *Single infusion of hypertonic salt solution.* Since the quantity of fluid administered is an important factor to be considered when comparing the value of blood substitutes on shock prevention, a series of experiments was performed in which small amounts of highly concentrated salt solution were used. These solutions were given intravenously at a rate not exceeding 0.5-1.0 cc. per minute.

In the first of these experiments, 20 cc. of a 20 per cent salt solution were given at the time of the secondary pressure fall to 12 dogs. Six of these remained symptom free and survived, and the other 6 died in shock (table 2). The pre-injection pressure levels were of the same order in the animals which died and those which survived. The infusion caused an immediate hemodilution, which, however, was less marked and of shorter duration than that which followed use of either plasma or gelatin (table 3). Owing to the intense thirst aroused by the injection of the strong salt solution, water was allowed at the end of the

8th hour instead of waiting a full 24 hours as in the plasma and gelatin experiments. The ingestion of water did not seem to influence the results.

7. *Intermittent infusion of hypertonic salt solution, 20 per cent.* Twenty cubic centimeters of a 20 per cent salt solution was divided into 5 doses, and given intermittently over the first seven hours following trauma to 12 dogs. Six of these showed no signs of shock, and 6 died in the usual time interval. There was, therefore, no indication that the intermittent saline injection was more efficacious than the single infusion. Hemodilution was as large as that observed following either plasma or gelatin transfusions (table 3).

8. *Intermittent infusion of 30 cc. hypertonic salt solution, 15 per cent.* Fifteen traumatized dogs were given 5 intermittent injections of 6 cc. each of a 15 per cent salt solution over a 7 hour interval. Nine animals of this series died in shock, and 6 survived. This proved the least successful series. A possible explanation for the rather low survival rate may have been the deleterious effect of the high temperature and high humidity which prevailed in the laboratory at the time these experiments were performed.

DISCUSSION. In so far as the treatment of shock following release of leg constrictions is concerned, the efficacy of intermittent transfusions over single transfusions seems quite clear. Neither a single infusion of salt solution nor one of plasma can prevent shock (1). The same amount of plasma, divided into 5 equal doses, and given intermittently over a 7 hour period, is highly successful in protecting against shock. Likewise, a similar volume of a 5 per cent gelatin solution administered in similar fashion proved strikingly efficacious.

The shock following leg muscle trauma appears to be less responsive to transfusions. Once again the value of intermittent plasma transfusions over a single injection seems established, since in the former case 77 per cent of the animals survived, and in the latter but 20 per cent failed to show shock. The results obtained with gelatin are not so decisive however, for with both intermittent and single transfusions, the survival rate was of the order of 50 per cent. Since salt solution alone is also effective in about 50 per cent of the cases, it would be difficult, if not impossible, to differentiate between the gelatin and the salt effect in shock prevention in these experiments. Unlike the shock following leg constriction, that following leg muscle trauma seems less responsive to gelatin than to plasma transfusions.

The evidence seems clear that some degree of protection against shock which follows the type of muscle trauma employed is afforded by infusions of salt solution alone. A single large infusion seems as efficacious as repeated smaller injections, and a small volume of strongly hypertonic solution less valuable than a larger volume of isotonic salt solution. However, the experiments indicate that saline infusions are relatively ineffective in preventing shock when such infusions are started when the arterial pressure is very low and shock well advanced. On the other hand, saline infusions are apparently highly beneficial when given early and before the blood pressure has fallen markedly, i.e., before symptoms of shock appear. It seems not unlikely that large infusions of 1 per cent saline or 5 per cent gelatin, if given immediately following leg muscle

trauma, would effectively prevent shock in an even larger number of the traumatized dogs than the 50 per cent obtained in these experiments. This represents a positive effect since in a series of untreated controls but 4 of a total of 37 dogs survived.

SUMMARY AND CONCLUSION

1. Twenty five cubic centimeters per kilogram of 5 per cent gelatin in physiological salt solution given intermittently over a 7 hour period at the rate of 5 cc. per kgm. prevented shock in all of 14 dogs following release of leg tourniquets.

2. The intermittent method of transfusing small amounts of plasma is much more effective in preventing shock following leg muscle trauma, than is a single large transfusion. Ten of 13 dogs did not show symptoms of shock after trauma when given plasma intermittently whereas but 2 of 10 dogs survived when a single large transfusion was employed.

3. Both 5 per cent gelatin in physiological salt solution and salt alone either in isotonic or hypertonic solution given as a single injection or intermittently over 7 hours led to survival of approximately 50 per cent of the traumatized animals.

4. Saline infusions are relatively ineffective in preventing shock induced by leg muscle trauma when such infusions are started when the arterial pressure is low and shock well advanced. However, they are apparently highly beneficial when administered before shock symptoms have appeared.

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THE ECCENTRICITY OF STANDING AND ITS CAUSE¹

F. A. HELLEBRANDT, BETTE G. NELSON AND ELEANOR M. LARSEN

From the Departments of Physiology and Physical Medicine, University of Wisconsin

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The early investigators in the field of animal mechanics assumed that in the well-formed individual the vertical projection of the center of gravity of the body as a whole must lie in the midsagittal plane (Schäfer, 1900). Thus the human body in the *normal Stellung* of Braune and Fischer (1890) was represented as passively poised over the ankle joint in a strictly symmetrical posture. Although it is well recognized that gravity is the chief deforming force affecting the vertical alignment of man, the magnitude and disposition of the gravitational rotatory stresses have been little studied, and direct experimental confirmation of the symmetry of standing is difficult to find. We have observed that the vertical projection of the center of gravity tends to fall slightly to the left and behind the geometric center of the total supporting base (Hellebrandt and Braun, 1939; Hellebrandt and Fries, 1942). This posterosinistral stance eccentricity occurs with sufficient frequency to suggest that the deviation may have biological significance. We found it to be characteristic of about 80 per cent of the subjects thus far studied.

Anatomic and physiologic asymmetries are frequent in Nature. The population of the universe appears to consist mainly of right-handed individuals. The crown whorl of head hair, when viewed from above, has a clockwise twist in the great majority of persons (Newman, Freeman and Holzinger, 1937), but the fetal rotation of the gut is counter-clockwise in direction and duodenal hernias occur on the left in 76 per cent of the reported cases (Gushue-Taylor and Hayward, 1942). There is a preponderance of curvatures of the spine convex to the left (Kuhns, 1938), and thromboses of the left iliac vein (McMurrich, 1908). Inequality in breast size has been reported, the left commonly being the larger (Gray, 1942). There is usually some lateral torsion of the uterus from left to right (Schumann, 1936). This is exaggerated during gestation and may reach 70 degrees. Occasionally the torsion may be in the opposite direction but statistics show that it occurs from left to right in 80 per cent of cases (Stander, 1941). Searching for an explanation of stance asymmetry we postulated that this might be compensatory for a right-sided morphological preponderance associated with anterodextral functional limb preference (Hellebrandt and Fries, 1942). The object of this study was to determine whether there is significant antecedence in the volitional use, and difference in strength and size of the limbs of the two sides of the body.

METHODS. 1. *Limb preference.* The first observations of limb preference

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were made by the method of Irwin (1938). They were subsequently confirmed, using an electronic precedence indicator devised and constructed by Gilson (1943). The subject, isolated in a dark room, was seated with the hands or feet resting on telegraph keys. The operator, from an adjoining room, controlled a bank of four colored lights, suitably dimmed and adjusted in front of the subject at eye level. The test was of the complex dilemma type. Whenever a predetermined combination of lights flashed, the subject responded by lifting the hands or feet from the keys, replacing them immediately. Six light combinations were employed. The order in which they were administered was standardized by random choice, arrived at by throwing a die to each numeral of which one light combination was assigned. The series consisted of 83 light flashes containing 25 of the stimulating combinations in chance distribution. The series was administered in four consecutive bouts to estimate the preference of the upper and lower extremities, each thus responding 100 times. The keys upon which the hands or feet rested controlled thyatron tubes which operated electro-magnetic counters. If one limb was raised at least 10 microseconds before the other, its precedence was recorded on the appropriate counter.

2. *Strength.* Strength tests are notoriously unreliable. To minimize the influence of uncontrollable variations in response, four different tests were employed. The force of a maximum effort was measured with a standard grip dynamometer. Martin's strength test (1918) was modified and used to estimate resistance to a gradually increasing force. To expedite the administration of the test, equipment was constructed which allowed firm immobilization of the subject during the application of the pull. A padded sling was fixed at right angles to the part to be tested and attached to a spring scale. To permit accurate adjustment of the angle of pull, the scale was suspended at the appropriate level from a ridge pole which could be swung through an arc of 180 degrees. A steady, gradually increasing pull was supplied by a half horse power a.c. motor. At the instant the resisting muscular contraction was overcome, the motor was switched off and the scale read. The muscle groups studied were those which produce protraction and retraction of the shoulder girdle, flexion and extension of the forearm, hip joint extension, abduction and adduction, and knee joint extension. The maximum resisting force which could be summoned was measured.

Repeated contractions may give a better estimate of muscle strength than a single maximum positive or resistive effort. A comparative study was made of the strength of the third digit flexors of the right and left hands using the Mosso ergograph. The two sides were tested alternately. The rhythm of contraction was set with a metronome and kept constant. The total number of trials was also kept constant. The subject made 30 maximum efforts in 60 seconds. The initial load was 1 kgm. This was augmented by 0.5 kgm. increments at successive trials to 4, 4.5 or 5 kgm. depending upon the strength of the subject. A kymograph record of the fatigue curve was obtained and the total distance through which the load was lifted was measured.

A similar strength test was devised to appraise the staying power of the

lower extremities. The subjects pedaled the electrodynamic brake bicycle ergometer first with one and then with the opposite leg (Kelso and Hellebrandt, 1934). The free lower extremity was comfortably supported during the exercise. A clamp attached to a rigid vertical support stabilized the pelvis during the unilateral pedaling. Alternate subjects rode first with opposite legs. After a suitable recovery period the activity was repeated on the opposite leg. The subject rode against a heavy load at a comparatively slow rhythm. The rate of working ranged from 309 to 740 kgm.-m/min. at an average r.p.m. of 47. The majority selected 500 kgm.-m/min. Each trial was carried to exhaustion. To assist in the differentiation of spurious from physiologic end-points of fatigue, heart rate and blood pressure responses to the exercise were measured. Observations were made at 60 sec. intervals and continued through the whole of the exercise and recovery periods after the subject had been carefully stabilized in recumbency. The work was hard. Cramps of the leg muscles occurred frequently during or at the cessation of the exercise. Heart rates occasionally reached 200/min. The systolic blood pressure did not exceed 190 mm. Hg. The duration of exercise was the independent variable. If the cardiovascular responses to the shorter ride were comparable to those of the longer, it was concluded that an all-out effort had been made on both trials, and the difference in staying power was not of psychic origin.

3. *Limb volume.* To gauge morphologic differences in size which might account for stance eccentricity the volume of the appendages was measured, immersing the limbs in water at approximately skin temperature and estimating the quantity of fluid displaced. Since superiority in functional capacity may be associated with improvement in blood supply and an increase in the size of the peripheral vascular bed an estimate was also made of the fluid accumulating in each limb under the influence of the hydrostatic effect of gravity when allowed to act for a given period of time, 20 minutes, under strictly standardized conditions.

4. *Morphologic symmetry.* As a final test of symmetry, differences in the weight of the two sides of the body were observed in recumbency on a balance board which permits the location of the center of gravity in a plane passing from head to foot. The subject was carefully balanced on a movable platform suspended from knife edges with gentle traction applied to head, wrists and ankles through equalizing yokes. A counter-weight was used to nullify the distorting effect on the center of gravity produced by platform movement. A sight line was strung from head to foot in the plane of the knife edges and the subject was photographed from above. The distance between the sight line and selected anatomical points could then be measured.

RESULTS AND THEIR INTERPRETATION. The subjects of the investigation were normal young adult women, professional students in physical education who participate in a variety of symmetric and asymmetric activities. They were accustomed to the severe exercise of competitive athletics, were familiar with laboratory procedures and could be relied upon to put forth a maximum physical effort. Forty-seven women acted as the subjects for the limb preference

studies. The remaining observations were made on a group of 20, each being a subject for the series of 8 experiments. The results are summarized in table 1.

1. *Limb preference.* The limb preference data yielded 5400 responses for the hands and a similar number for the feet. The right hand responded first in 49 per cent of the total number of trials, the left in 51 per cent. The difference between the two is small. The same is true of the foot responses, except that the preference was reversed, and the difference between them was slightly greater, 53 and 47 per cent respectively. In the analysis of the group data a slight preponderance of choice fell to the right foot and to the contralateral or opposing hand, as though the preferred lower limb were being maintained in a position of readiness for action with the center of weight eccentric in a counterbalancing position.

TABLE 1

Mean preference, strength and size of the limbs and the difference between the right and left sides

	RIGHT			LEFT			DIFFERENCE		UNITS
	M	SD	V	M	SD	V	D	CR	
Arm preference....	47.50	28.58	60.17	52.80	27.69	52.44	5.30	0.91	% total trials
Leg preference....	52.59	32.85	62.46	47.69	32.43	68.00	4.90	0.73	% total trials
Mosso ergograph.	112.25	61.75	55.01	102.25	60.25	58.92	10.00	1.33	kgm.
Grip strength.....	33.76	4.75	6.39	27.22	4.10	6.85	6.54	4.43	kgm.
Martin test.....	445.53	80.61	18.09	431.47	74.52	17.27	14.06	0.53	lb.
Bicycle time.....	12.42	7.92	63.77	10.95	6.51	59.45	1.47	0.61	min.
Bicycle work.....	5686.67	3318.62	58.36	5001.50	2750.69	55.00	685.17	0.67	kgm.-m./min.
Arm volume.....	1.54	0.30	19.48	1.45	0.33	22.76	0.09	0.92	l
Leg volume.....	4.80	0.81	16.87	4.73	0.88	18.60	0.07	0.27	l
Arm edema.....	82.18	52.51	63.90	74.55	36.76	49.31	7.63	0.39	cc.
Leg edema.....	254.54	126.02	49.51	235.91	168.11	71.26	18.63	0.29	cc.

2. *Strength.* The right grip strength was significantly stronger than the left. This was true of all but one subject, who was left-handed. The average right grip was 34 kgm. strong as compared with 27 kgm. on the left. Thus the socially dominant hand was 24 per cent stronger than the contralateral extremity and 55 per cent of the total grip strength resided on that side. A total of 272 muscle groups were measured as to strength by the Martin test. The maximum resisting force developed on the two sides was practically identical, 50.80 per cent being distributed to the right and 49.20 per cent on the left. Thus the right side was only 3 per cent stronger than the left. Dawson (1935) states that the difference in strength between the two sides of the body is very small indeed. In 1918 Martin made a study of muscular strength and symmetry and concluded that the difference in the strength of the two sides of the body, when

measured by his method, was neither great enough nor sufficiently constant to involve serious error if the two sides were assumed to be equal.

The series of experiments with the Mosso ergograph yielded 262 kymographic fatigue curves. The right and left strength were again very nearly the same. The right middle digit was 10.08 per cent stronger than the same finger of the contralateral limb. The mean duration of the unilateral exercise of the bicycle ergometer on the right exceeded the endurance on the left by 13.5 per cent. The average total work done was well over 10,000 kgm.-m, 53.20 per cent being contributed by the right extremity and 46.80 per cent by the left.

3. *Limb volume.* The stronger right arm displaced more water than the left, the difference being 6 per cent. The legs were more nearly equal in size. The difference in the amount of water displaced was only 1.5 per cent greater on the right than on the left. These slight differences in limb volume were exaggerated when the stasis and edema of hydrostatic origin were measured. The findings suggest that greater strength may indeed be associated with an increased capacity of the vascular bed. The accumulation of five or six hundred cubic centimeters of fluid unequally distributed between the appendages of the two sides thus adds itself to the small differences in muscle mass associated with asymmetry of strength and limb preference. As seen from an examination of the critical ratios in the table, the only statistically significant difference between the right and left sides is in grip strength. This is perhaps to be expected because of the lack of homogeneity in this small group. The average weight of the subjects was 138.82 lb. but this important variable ranged from 106 to 173 so that great differences in strength and limb volume must follow. It is suggested that the sum of the various differences may reach proportions of significance to the mechanisms concerned with the maintenance of balance. It may be a matter of no great physiological moment to cope with one, whereas in the aggregate a group of small asymmetries may make themselves felt, especially if they fall persistently to the same side. Thus conceived, the data take on increased significance.

4. *Morphologic symmetry.* When the right and left sides of the body were compared with the subject in recumbency on the balance board, 85 per cent were observed to be heavier on the right side than on the left. Only one appeared to be strictly symmetrical. How much of this is due to differences in muscle mass and how much to inequalities in visceral weight is open to speculation. Lyman (1942) reports the weight of muscle in rats to be heavier on the right in 94 per cent of the experimental number. This raises the question as to whether human asymmetry is inherited from the quadruped.

If the right side of the body is, in general, stronger and heavier than the left, it remains of interest to consider why the average center of gravity is eccentric to the contralateral instead of the homolateral side. This may be a simple overcompensation phenomenon, acting in the anteroposterior as well as the transverse vertical plane. Standing is not a static phenomenon. It is, in reality, movement upon a stationary base. In stable and physiologically well poised individuals, postural sway may be virtually insensible but this is by no

means invariably true. That postural sway is inseparable from the upright stance has been abundantly recognized and extensively studied since the pioneer observations of Vierordt (1862). It follows that the center of gravity of the body as a whole must also shift incessantly during standing.

To suppose that the average position of the trajectory of the incessantly shifting center of weight will plumb over the exact center of the base is expecting much of the automatisms known to control posture. Undercompensation would be unbiological since biped standing, being a basic requisite for normal life and activity, is well protected. Overcompensations, on the contrary, are commonly observed in the normal functioning of all organ systems. Since the center of gravity falls well in front of the axis of rotation of the ankle joint, gravitational rotatory stresses keep the leg constantly in an unbalanced position. The stresses tending to tip the body forward are equilibrated by the antigravity extensors. If they overshoot their mark in efforts of protection, the average position of the observed center of gravity will fall behind the geometric center of the base. Similarly, the heavier right side of the body unbalances the stance in the transverse vertical plane, giving rise to a second rhythmic series of myotatic reflexes in muscles which respond in a manner destined to equilibrate the disturbing force. Each time a barrage of proprioceptive impulses elicited by stretch associated with postural sway impinge upon cord centers, the motor impulse called forth more than meets the unbalancing force and the average center of gravity falls off-center to the contralateral side.

SUMMARY AND CONCLUSIONS

A series of experiments designed to yield quantitative estimates of right and left-sided differences in size, strength and limb preference were performed on a small group of young adult women in an effort to elucidate the mechanism of the slight posterosinistral eccentricity of the vertical projection of the center of gravity of the body as a whole which characterizes the upright stance of 80 per cent of normal subjects. The evidence substantiates the following conclusions:

1. Morphologic and functional asymmetries occur in limb preference, volume and strength.
2. Although most of the observed asymmetries are too small to have statistical significance they constantly favor the right side.
3. It is suggested that in the aggregate these small dextral asymmetries in functional capacity associated with like differences in strength and size have the effect of a slightly eccentric counterweight on the incessantly shifting rotatory moments acting on the joints of the weight-bearing skeletal parts. The autonomous equilibrating muscular contractions called forth overcompensate for the force of this eccentric weight and the anteriorly unbalanced position of the leg by an amount great enough to result in a slight eccentricity in the location of the mean vertical projection of the center of weight in a position contralateral to the sum of the unequal stresses.

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especially for his design and construction of the modification of the Martin Strength Test apparatus described in this paper; to Dr. W. E. Gilson for his development of the electronic precedence indicator; and to E. Betts, P. King, D. Lybarger and M. Merrill for technical assistance in the conduct of the experiments.

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ON THE CONSTITUTION OF PROTHROMBIN¹

ARMAND J. QUICK

From the Department of Pharmacology, Marquette University School of Medicine, Milwaukee

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Prothrombin is recognized entirely by its activity. Little is known of its actual composition except that it is either a protein containing about 4 per cent carbohydrate (1), or is closely associated with such a protein. The possibility that prothrombin may be a complex like complement made up of several components has not been widely recognized since no experimental data in its support have heretofore been offered. It is the purpose of this paper to present a number of simple experimental findings which can best be co-ordinated and explained by postulating that prothrombin is composed of two essential and separable factors which appear to be combined with calcium.

Evidence of two components in prothrombin. When oxalated human plasma in an unstoppered container is placed in a refrigerator, the prothrombin as measured by the author's method (2) progressively decreases as illustrated in table 1. It has previously been shown (3) that the destruction of prothrombin can be hastened in human blood by heating the plasma to 38°C. and passing a current of air through it. It appears that oxidation is the cause of the diminution of prothrombin. This is supported by the finding that the disappearance of prothrombin can be prevented or retarded by a layer of carbon dioxide gas above the plasma.

The feeding of toxic sweet clover hay or its toxic principle, now known to be 3,3 methylene-bis-(4 hydroxy coumarin) (4), causes a profound reduction of the prothrombin of the blood. The typical course of the hypoprothrombinemia in dicumarol² poisoning is presented in table 2.

Since the prothrombin of the plasma is diminished both after storage and in dicumarol poisoning, it is to be expected that when the two types of plasma are mixed, the blended plasma should show a prothrombin content corresponding to the average of the two plasmas. Surprisingly this is not the case as experiment 1 demonstrates.

The fact that mixing the two plasmas, both of which showed a marked reduction of prothrombin, caused a restoration of the prothrombin above the normal level of human plasma, indicates that the diminution of prothrombin in the two types of plasma is not identical. The simplest explanation is that prothrombin is composed of two factors or components, one of which is labile in vitro and disappears when blood is stored, while the second becomes diminished when the animal is poisoned with dicumarol. For simplicity the first factor is designated as component A, and the second—component B.

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² The dicumarol used was kindly furnished by Eli Lilly and Company.

Component A. This factor is demonstrable by its ability to restore the prothrombin time of stored plasma. In testing for its presence or estimating its concentration, component B must first be removed. The latter is completely adsorbed by aluminum hydroxide, which does not remove component A. The test is carried out as follows: nine volumes of oxalated plasma are mixed with one volume of aluminum hydroxide cream. The mixture is incubated for 15

TABLE 1
Increase of prothrombin time in stored plasma

AGE OF PLASMA	PROTHROMBIN TIME (SECONDS) HUMAN PLASMAS							
	I	II	III*	IV	V	VI	VII	VIII†
<i>days</i>								
	12	11½	12	11½	11½	11½	11½	11½
1	14	16	15	12½	13½	12	12	12
2	16	17	17	14	15½	13	13½	12
3	20	19	21	14½	16	14½	15	12
4	22	21	23	16	19	17	15½	12
5	26	27	26	18	23	20	16	12½
6	31	33	31	19	27	27	22	12½

* The first three specimens show the rate of decrease of prothrombin usually observed during the winter months.

† Specimens 7 and 8 are from the same subject, but 8 was covered with a layer of carbon dioxide and stoppered.

TABLE 2
*Increase of prothrombin time after feeding dicumarol**

TIME	PROTHROMBIN TIME (SECONDS)	
	Rabbit	Dog
<i>days</i>		
	6 (100)†	6 (100)
1	12 (20)	11½ (20)
2	19½ (5)	20 (5)
3	44 (1½)	41 (1½)
4	110 (½)	43 (1½)
5	195 (>½)	150 (½)
6	720 (>½)	360 (>½)

* Ten milligrams of dicumarol per kilogram of body weight was fed daily.

† The figures in parenthesis are the concentration of prothrombin in per cent of normal.

minutes at 37°C. and stirred frequently. The aluminum hydroxide is removed by centrifugation. To 3 volumes of stored human plasma is added 1 volume of the "alumina plasma" which is to be tested. For the determination, 0.1 cc. of the mixed plasma and 0.1 cc. of thromboplastin are transferred to a small test tube, and 0.1 cc. of 0.02 M calcium chloride forcefully blown in to obtain instantaneous mixture. The reaction is carried out at 37°C. and the coagulation time accurately measured with a stop watch.

It has been found that the content of component A in dog and rabbit blood is the same whether component B is removed physiologically by means of dicumarol or chemically with aluminum hydroxide as shown by experiment 2.

The concentration of component A appears to be much higher in dog and rabbit plasma than in human, but considerable fluctuation of factor A occurs in the latter. See experiment 3.

EXPERIMENT 1

The decrease of prothrombin in stored plasma and after feeding dicumarol

	PROTHROMBIN TIME	PROTHROMBIN CONCENTRATION†
	seconds*	
Human plasma I (stored 8 days).....	45	6
Dog plasma II (after feeding dicumarol).....	180	>1
Plasma I + Plasma II (equal volumes of each).....	10	<100

* The clotting time of 0.1 cc. of oxalated plasma mixed with 0.1 cc. of thromboplastin and 0.1 cc. of 0.02 M calcium chloride.

† In terms of per cent of normal on the basis of the prothrombin level in human plasma.

EXPERIMENT 2

Removal of component B from plasma with aluminum hydroxide and by feeding dicumarol

	PROTHROMBIN TIME
	seconds
Human plasma I (stored 5 days).....	24
Dog plasma II (treated with $\text{Al}(\text{OH})_3$).....	∞
Dog plasma III (after feeding dicumarol).....	180
0.3 cc. of plasma I + 0.1 cc. of plasma II.....	10
0.3 cc. of plasma I + 0.1 cc. of plasma III.....	10

EXPERIMENT 3

The concentration of component A in dog and in human plasma

	PROTHROMBIN TIME
	seconds
Human plasma I (stored 6 days).....	37
Dog plasma II (treated with $\text{Al}(\text{OH})_3$).....	∞
Human plasma III (treated with $\text{Al}(\text{OH})_3$).....	∞
0.3 cc. of plasma I + 0.1 cc. of plasma II.....	10
0.3 cc. of plasma I + 0.1 cc. of plasma III.....	25

It will be observed that the concentration of component A is much higher in dog than in human plasma. In fact the addition of dog or rabbit plasma, from which component B has been removed, to human plasma (either fresh or stored) reduces the prothrombin time to 9 or 10 seconds. This is shorter than that of normal plasma which is 11 to 12½ seconds. Whether this is to be interpreted that human plasma contains less than the optimum amount of component A in relation to its concentration of the B factor is a subject of further inquiry. The

rate of disappearance of component A from human plasma varies considerably. It is not possible at present to state whether this is due to a greater initial concentration or to variations in stabilizing factors of the plasma. It appears that there is a seasonal variation, but this requires further study.

Component A is destroyed by heat. If either alumina or dicumarol plasma is heated at 60°C. for 15 minutes, little active component A remains as shown by experiment 4.

Both component A and B are to a certain degree group specific. Thus, component A of chicken plasma does not reduce appreciably the prothrombin time

EXPERIMENT 4

The effect of heat on components A and B

	PROTHROMBIN TIME
	seconds
Human plasma (stored 9 days).....	50
Dog plasma II (treated with $\text{Al}(\text{OH})_3$).....	∞
0.3 cc. of plasma I + 0.1 cc. of plasma II.....	10
0.3 cc. of plasma I + 0.1 cc. of heated plasma II*.....	30
0.3 cc. of heated plasma I* + 0.1 cc. of plasma II.....	∞

* Incubated at 60°C. for 15 minutes.

EXPERIMENT 5

Group specificity of components A and B

	PROTHROMBIN TIME
	seconds
Human plasma I (stored 7 days).....	38
Chicken plasma II (treated with $\text{Al}(\text{OH})_3$).....	∞
0.3 cc. of plasma I + 0.1 cc. of plasma II.....	32*
Chicken plasma III (stored 6 days).....	42
Dog plasma IV (treated with $\text{Al}(\text{OH})_3$).....	∞
0.3 cc. of plasma III + 0.1 cc. of plasma IV.....	33†

* Rabbit brain thromboplastin was used. With chicken brain thromboplastin the prothrombin time was 105 seconds.

† Chicken thromboplastin was used. With rabbit brain thromboplastin the prothrombin time was 180 seconds.

of stored human plasma; and alumina rabbit plasma only slightly lowers the prothrombin time of stored chicken plasma (expt. 5).

The stability of component A in true or unmodified plasma. Chicken or goose plasma which can be kept liquid without the addition of a decalcifying agent shows little or no diminution of prothrombin when stored, whereas the oxalated or citrated plasma loses its prothrombin, or more accurately its component A, promptly, as shown in experiment 6.

This experiment clearly demonstrates that component A is stable when the plasma is in its native or unmodified state, whereas in oxalated or citrated plasma it is easily destroyed. This suggests that in blood the two components

are bound by calcium, and that they are thus protected against external factors such as oxidation.

Component B. As already stated, it is this factor which diminishes when an animal is fed dicumarol. Preliminary studies suggest that a deficiency of vitamin K likewise produces only a reduction of component B. Thus, in a patient with obstructive jaundice, a definite decrease in the latter was found.

Component B is destroyed by heating to 60°C. (see expt. 4). It is group specific as demonstrated in experiment 5. Aluminum hydroxide removes the component completely from oxalated plasma, but apparently does not adsorb it from true or unmodified plasma in which the calcium has not been removed and the prothrombin complex has been allowed to remain intact. The evidence for this was obtained by adding just sufficient heparin to rabbit blood to prevent

EXPERIMENT 6

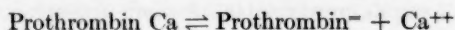
A comparison of the stability of prothrombin (or component A) in oxalated and in unmodified plasma

Unmodified chicken plasma....	0.1 cc.	Oxalated chicken plasma.....	0.1 cc.
Saline solution.....	0.1 cc.	Chicken thromboplastin.....	0.1 cc.
Chicken thromboplastin.....	0.1 cc.	Calcium chloride 0.02 M.....	0.1 cc.
Clotting time.....	11 sec.	Clotting time.....	11 sec.
	After 3 days storage*		
	12 sec.		18 sec.
	After 7 days storage		
	10½ sec.		32 sec.
Unmodified goose plasma.....	0.1 cc.	Oxalated goose plasma.....	0.1 cc.
Saline solution.....	0.1 cc.	Chicken thromboplastin.....	0.1 cc.
Chicken thromboplastin.....	0.1 cc.	Calcium oxalate 0.02 M.....	0.1 cc.
Clotting time.....	9½ sec.	Clotting time.....	10 sec.
	After 4 days storage		
	10 sec.		32 sec.

* Blood was kept in small unstoppered test tubes coated with collodion. The temperature of storage was 10°C.

coagulation. Such plasma will on the addition of thromboplastin still coagulate readily. To 9 volumes of the heparinized plasma, 1 volume of aluminum hydroxide cream was added, and the mixture incubated at 37°C. for 15 minutes. As a control, a sample of the heparinized plasma was oxalated before treatment with aluminum hydroxide. The results are recorded in experiment 7.

The calcium factor. In a previous publication the author (5) postulated that prothrombin is a calcium compound which is slightly ionized.



This equilibrium is expressed by the equation:

$$\frac{\text{Prothrombin}^- \times \text{Ca}^{++}}{\text{Prothrombin Ca}} = K$$

Sodium oxalate or citrate inhibits coagulation by depressing the calcium ions. The action of sodium oxalate solution is relatively slow, probably because of the lag with which the equilibrium between soluble and insoluble calcium oxalate is established. It was found that sodium citrate acts promptly and therefore is better suited to study the depression of calcium ions and its effect on prothrombin.

Experimental. Blood was drawn directly into a test tube under a layer of mineral oil. The test tube was coated with collodion and the connecting glass tubing with paraffin. The test tube was immersed in ice, and carbon dioxide run through the apparatus just prior to the collection of the blood. By exercising great care in preventing the blood from coming in contact with air or glass surface, coagulation could be retarded 6 hours or more.

From the findings recorded in table 3, it can be seen that more citrate is needed to suppress the coagulation of rabbit blood than of human blood. This is to be

EXPERIMENT 7

The action of aluminum hydroxide on component B in decalcified and non-decalcified plasma

Heparinized rabbit plasma*.....	0.1 cc.	Heparinized rabbit plasma treated with $\text{Al}(\text{OH})_3$	0.1 cc.
Saline solution.....	0.1 cc.	Saline solution.....	0.1 cc.
Thromboplastin.....	0.1 cc.	Thromboplastin.....	0.1 cc.
Clotting time.....	12 sec.	Clotting time.....	16 sec.
Oxalated heparinized plasma†..	0.1 cc.	Oxalated heparinized plasma treated with $\text{Al}(\text{OH})_3$	0.1 cc.
Thromboplastin.....	0.1 cc.	Thromboplastin.....	0.1 cc.
Calcium chloride 0.02 M.....	0.1 cc.	Calcium chloride 0.02 M.....	0.1 cc.
Clotting time.....	13 sec.	Clotting time.....	∞

* The blood contained 6 Toronto units (0.006 mgm.) of heparin per 5 cc.

† The blood contained 10 Toronto units (0.01 mgm.) of heparin per 5 cc.

expected since the author (6) has found by various methods that rabbit blood contains five times more prothrombin than does human blood. If it is assumed that only prothrombin Ca is active, i.e., is convertible to thrombin, one can conclude that prothrombin can be estimated at least roughly by titration of the plasma with sodium citrate. Thus, if 0.02 cc. of 0.1 M sodium citrate is added to 0.5 cc. of human plasma, the prothrombin time is increased to 15 seconds which according to the author's prothrombin curve indicates a 50 per cent reduction of the prothrombin. An inspection of the data of table 3 shows that if the calcium ion concentrations be plotted against the prothrombin times, a hyperbolic curve is obtained resembling the typical and familiar prothrombin curve. The exact mathematical treatment of the data will be reserved for a future publication.

It is interesting that Ransmeier and McLean (7) studying the effect of the calcium ion concentration on the coagulation time of citrated plasma likewise

obtained a rectangular hyperbola curve which corresponded to the empirical equation: $t = \frac{K}{Ca^{++-m}} + n$ (t = clotting time and K , m and n = constants).

The prothrombin curve, it will be recalled follows the equation $t = \frac{K}{c} + a$ (c = concentration of prothrombin; K and a = constants). On the assumption that the amount of active prothrombin is proportional to calcium ion concentration, the similarity of the two curves becomes apparent. Ransmeier and McLean did not control the thromboplastin, which conceivably can vary in dog and human blood; nevertheless their finding that human plasma requires a higher concentration of calcium ions (0.35 mM per liter of plasma) than dog plasma (0.28 mM) is entirely in accord with the present findings.

TABLE 3

The inhibition of coagulation (in the presence of excess thromboplastin) by sodium citrate

PERIOD OF INCUBATION	SODIUM CITRATE 0.1 M ADDED TO 5 CC. OF PLASMA										
	0	0.01 cc.	0.02 cc.	0.03 cc.	0.04 cc.	0.05 cc.	0.06 cc.	0.07 cc.	0.08 cc.	0.09 cc.	
	Clotting time (seconds)										
10 sec.	5½	6	6	6½	9	10	15	22	73	120	Rabbit plasma
11 min.	*	6	6	6½	8½	11	16	33	80	150	
3 min.		*	*	7	9	11	16	30	80	160	
10 min.				*	*	*	16	32	90	160	
10 sec.	11	12	15	36	75	210	480				Human
1 min.	10½	12	15	39	100	210	†				
3 min.	*	11½	15	30	95	220					
10 min.		*	15	32	80	190					

The clotting time was determined by mixing 0.1 cc. of the citrated plasma with 0.1 cc. of saline solution and 0.1 cc. of thromboplastin emulsion.

* Plasma clotted spontaneously.

† Only a few shreds of fibrin but no solid clot formed.

DISCUSSION. The data presented support the concept that prothrombin is a complex composed of three factors: component A, component B and calcium. By the removal of calcium the prothrombin is dissociated, but on the readdition of ionic calcium a resynthesis of active prothrombin immediately occurs. A diminution of any one of these three factors causes a decrease of the prothrombin as measured by the one-stage method. There is suggestive evidence that component A is somehow related to the oxidation and reduction system of the blood; whereas component B appears to be the body of the prothrombin complex, the factor which disappears in dicumarol poisoning and perhaps in vitamin K deficiency. Further work, however, is required before definite statements concerning these factors can be made.

The new concept is of theoretical importance, especially in emphasizing that biological agents may be complexes that are easily dissociated and resynthesized. It is to be remembered that complement which bears many resemblances to

prothrombin is composed of several well recognized components. The difficulty of isolating such a biological agent as a chemically pure substance is easily comprehended.

Several practical considerations arise from the hypothesis that prothrombin is a three-component complex. The first concerns the quantitative determination of prothrombin. Little is known concerning the quantitative relationship of component A to B, and therefore it seems hazardous to depend on results obtained by high dilution of plasma since one must assume that prothrombin which is dissociated in decalcified plasma is on recalcification resynthesized, i.e., components A, B and calcium are recombined. The use of plasma treated with aluminum hydroxide as a prothrombin-free medium may, as seen in the light of these new developments, lead in certain experiments to serious errors. The author in developing the prothrombin curves fortuitously usually employed human alumina plasma which is relatively low in component A. Fortunately, too, the alumina plasma was generally used as a diluent; and at high dilution of component B, the effect of component A is slight. Nevertheless, aluminum hydroxide-treated plasma can no longer be considered merely as being prothrombin-free—cognizance must be taken of its content of component A.

Views on the use of stored plasma for transfusion must be modified. Heretofore, such plasma was considered unsuitable for treating hypoprothrombinemia. Since stored plasma only loses component A and as only component B is depleted in dicumarol poisoning, the employment of stored plasma should be equally as effective as fresh plasma. This is of practical importance since patients receiving dicumarol may precipitously develop a hemorrhagic condition that demands an emergency transfusion.

With the realization that prothrombin is composed of several factors, a clearer and fuller understanding of the hypoprothrombinemias should be attained and a more concise classification should be possible. At present all the cases of clinical hypoprothrombinemia appear to be deficiencies of component B. Recently the author discovered a constant prothrombinopenia in an otherwise entirely normal young adult. The prothrombin remains at 45 per cent of normal and is not influenced by the administration of vitamin K. A decrease of component B was found to be responsible for the low prothrombin.

Although depletion of component A has been observed only *in vitro*, it is entirely probable that clinical hypoprothrombinemia due to lack of this factor may occur. Preliminary studies on chloroform poisoning in dogs have yielded results that indicate a temporary fall of both components.

SUMMARY

1. Experimental findings are presented which indicate that prothrombin is composed of calcium and two separable components designated A and B.
2. Component A disappears from oxalated plasma when stored in a refrigerator—it is presumably destroyed by oxidation. It is heat labile and to a certain degree group specific. In unmodified plasma, it does not diminish and therefore it can be concluded that factor A when present in the intact prothrombin complex is stable.

3. Component B disappears in the plasma of animals poisoned with dicumarol. It is heat labile and is completely removed from oxalated plasma by aluminum hydroxide. This adsorbent does not remove the factor from unmodified plasma, thus indicating that when it is combined in the prothrombin complex, it is not adsorbed.

4. Decalcifying agents inhibit the coagulation of the blood by depressing the calcium ion concentration of the system:

$$\frac{\text{Prothrombin}^{\text{--}} \times \text{Ca}^{++}}{\text{Prothrombin Ca}} = K$$

More sodium citrate is needed to suppress the coagulation of rabbit than of human blood. This is in accord with the finding that rabbit blood contains more prothrombin than is found in human blood.

5. The importance of the concept that prothrombin is a complex of several components is discussed in relation to *a*, the quantitative determination of prothrombin; *b*, the use of stored plasma for transfusion in the treatment of hypoprothrombinemia from dicumarol poisoning, and *c*, the classification and more exact understanding of clinical hypoprothrombinemia.

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THE EFFECT OF EPINEPHRINE UPON FROG RENAL HEMODYNAMICS IN THE INTACT ANIMAL¹

ROY P. FORSTER

From the Department of Zoology, Dartmouth College, Hanover, N. H.

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One of the classical studies in the history of renal physiology is that of Richards and Schmidt (1) who by direct observation described the glomerular circulation of the frog and noted the effects of small amounts of epinephrine² upon it. More recently an indirect clearance method of following the renal blood flow in the intact animal has been introduced by Smith, Goldring and Chasis (2). The object of this present study is to attempt to validate the indirect clearance methods of renal function examination by comparing results obtained through their use with those noted directly by microscopic examination of the functioning kidney, especially with respect to the effects of small amounts of epinephrine on the renal circulation.

Richards and Schmidt exposed the kidneys of pithed frogs for microscopic examination by transmitted light and injected dilute solutions of epinephrine into the anterior abdominal vein. Following the injections the glomerular tuft increased in diameter, became crowded with cells, and the blood flow through its capillaries was slower. The results were such that they might be explained either by accelerated inflow of blood due to cardiac stimulation or by constriction of the efferent arteriole. The former explanation was eliminated by maintaining a constant blood flow through the kidney during the epinephrine injections (Richards, Barnwell and Bradley, 3). In the latter experiments the kidney was perfused through the aorta at a constant rate with whole blood or oxygenated Hamburger's solution after the gastrointestinal tract and spleen were excised, the renal circulation isolated, and the kidney exposed for direct microscopic examination. Addition of small amounts of epinephrine to the perfusate resulted in an increase in the size of the glomerular tuft and an increase in perfusion pressure which indicated efferent constriction. This conclusion was further substantiated by Hayman (4) who directly measured pressure within the glomerular capillaries of the living frog and found it to rise after epinephrine was introduced into the circulation in dosages similar to those which Richards and Schmidt found to cause an increase in the size of the glomerular tuft and decreased glomerular flow. Hence, by direct observation it was determined that small amounts of epinephrine caused constriction of the efferent arterioles with subsequent increase in intracapsular pressure and decrease in renal blood flow.

When the dose of epinephrine was increased Richards and Schmidt observed

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² Although epinephrine has been variously referred to as adrenalin and adrenin in the literature of renal physiology the former is used exclusively throughout this discussion.

that blood flow in the glomerulus as well as in its arteriole stopped, apparently due to constriction of the afferent arteriole. It was noted, however, that while stoppage may occur in one glomerulus, in another glomerulus close to it the blood may keep on flowing with unaltered rapidity.

METHODS. In these experiments large well nourished bullfrogs ranging in weight from 400 to 750 grams were used. Because of the difficulties involved in getting accurate urine collections from the bladder or cloaca the ureters were exteriorized and cannulated with several inches of fine silk catheter a day or more before the experiment. In some instances when a measurement of only arterial renal blood flow was desired the renal portal blood supply was tied off at the same time. Caution was used and this operation was done with practically no loss of blood.

The standard clearance procedures outlined by Smith (5, 6) were used. The diodrast clearance at low plasma concentrations was used to measure the minimal renal plasma flow (Smith, Goldring and Chasis, 2). The creatinine clearance was used to measure the rate of glomerular filtration (Forster, 7). In a few instances intermittence of glomerular activity under massive doses of epinephrine was studied and the methods discussed in a previous paper were employed (Forster, 8).

In an animal which weighed 480 grams (G_3) the following procedure was used in the conduct of an experiment. A priming dose of 15 mgm. of diodrast and 200 mgm. of creatinine in 15 ml. saline solution was injected into the dorsal lymph sac and the bullfrog was placed in a battery jar half full of water. One hour later the frog was tied supine upon a holding board and prepared for infusion. The saline infusion fluid contained 15 mgm. per cent diodrast and 200 mgm. per cent creatinine and was introduced at a steady rate into the dorsal lymph sac by means of the dropping mercury technique. Fluid thus introduced into the lymph sac very quickly entered the blood stream due largely to the pumping action of a pair of lymph hearts at the posterior end of the lymph sac on either side of the urostyle. After considerable preliminary trial and error it was found that the plasma diodrast and creatinine concentrations could be kept quite constant by controlling the rate of infusion and the concentration of these substances in the perfusate. After two hours of infusion the first blood sample (3.5 ml.) was taken from the ventricle and the first of a series of 3 fifteen minute urine collections from the cannulated ureters was started. At the end of the third control period 0.01 mgm. epinephrine in 1 ml. saline solution was injected into the dorsal lymph sac and then 8 more 15 minute urine collections were made until the experiment was terminated. A blood sample was taken at the middle of the experiment and another at the end of the last urine collection.

Coagulation of blood was prevented by the use of dry heparin in the syringe and blood was centrifuged immediately upon being withdrawn. Blood proteins were precipitated by the cadmium method of Fujita and Iwataka (9). Diodrast concentrations in plasma and urine were determined by Smith's modification of Alpert's method (10), and creatinine by the method of Folin and Wu (11).

RESULTS. The effects of varying amounts of epinephrine on renal hemodynamics in 18 different bullfrogs were studied, each experiment involving an

average of 12 urine collection periods. The effect of a small dose is illustrated in figure 1. The injection of 0.01 mgm. of epinephrine after three fairly constant control periods resulted in a marked fall in the renal plasma flow (maximal diodrast clearance) and a corresponding rise in the filtration fraction ($\frac{\text{creatinine clearance}}{\text{diodrast clearance}}$) with the result that the rate of glomerular filtration (creatinine clearance) remained constant. The response to epinephrine administration was very rapid and recovery was obtained after 90 minutes.

The effect of a massive dose of epinephrine (0.1 mgm. or more) is characterized by an initial drop in the rate of glomerular filtration, the renal plasma flow and the filtration fraction. Within 15 to 30 minutes, however, the filtration fraction very rapidly increases and the filtration rate comes back to normal. The renal plasma flow remains low and does not increase until the epinephrine effect wears off and the filtration fraction drops. Tubular glucose reabsorption studies indicate that glomeruli close down in proportion to the initial drop in filtration rate.

The results indicate that epinephrine at normal levels exerts its effect on frog renal hemodynamics simply by causing constriction of the efferent glomerular arterioles. Only at high and probably abnormal levels does it affect the afferent arterioles causing constriction and stoppage of some glomerular activity.

DISCUSSION. These results obtained by the indirect methods of clearance analysis stand in complete agreement with those obtained in Richards' laboratory by direct observation of the functioning frog kidney. This, we think, constitutes a distinct link in the chain of evidence indicating that clearances provide reliable estimates of the various aspects of renal activity.

It is interesting to note that small amounts of epinephrine have exactly the same effect on both the amphibian and mammalian kidney. Chasis, Ranges, Goldring and Smith (12) have demonstrated in man that the renal blood flow appears to be controlled predominantly by the efferent glomerular arterioles. This has the effect of maintaining a filtration rate independent of the renal blood flow because variations in blood flow are accompanied by a corresponding inverse change in filtration pressure and filtration fraction which tends to maintain a constant filtration rate. Despite the fact that the frog maintains a constant filtration rate under the influence of small amounts of epinephrine, it must be remembered that afferent control is an important factor in maintaining water balance in these animals. Unlike mammals, variations in urine flow in the frog and other primitive vertebrates are accomplished by variations in the rate of glomerular filtration and in the number of functioning glomeruli. These alterations in glomerular activity are controlled largely by the afferent arterioles (Richards and Schmidt; 1, Forster, 7, 8; Friedlich, Holman and Forster, 13). Amphibians in and out of water are exposed variably to conditions requiring very rapid water elimination or extreme water conservation. Here the glomerulus assumes its primitive function and is the primary factor in regulation of water balance in contrast to the state in mammals where variations in urine flow are dependent entirely upon tubular reabsorptive activity.

The filtration fraction of the animal represented in figure 1 is quite low compared to that observed in mammals largely because this animal had its renal

portal blood supply intact during the experiment; hence, its maximal diodrast clearance represents not only the arterial blood which was delivered through the glomerulus but that venous fraction which supplied the tubules directly. The filtration fraction was considerably higher when the renal portal supply was removed before the experiment. Under the latter conditions 12 to 15 per cent of the blood delivered to the kidney is filtered in comparison with only 6 per cent when the portal supply is intact. The diodrast clearance at low plasma level (1 to 3 mgm. per cent) with the portal supply intact is about 700 ml. per kgm. per hr. and about 350 with the independent venous circulation removed. Appar-

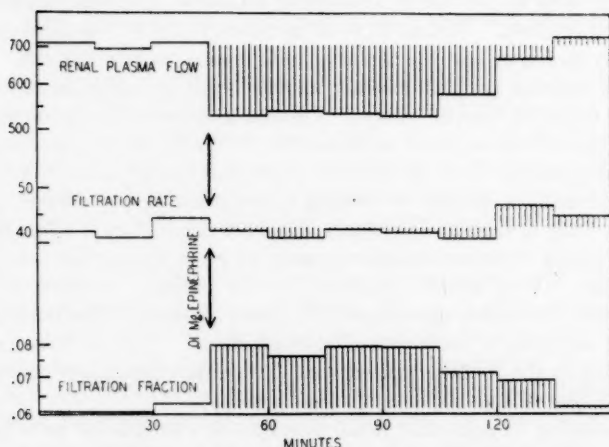


Fig. 1. A graphic illustration of the effect of 0.01 mgm. epinephrine on renal hemodynamics in a bullfrog (G_2) weighing 480 grams. The epinephrine was administered after 3 fifteen minute control periods and resulted in a marked drop in the renal plasma flow and a corresponding rise in the glomerular filtration fraction. The inverse relationship between plasma flow and filtration fraction indicates constriction of the efferent glomerular arterioles and results in maintaining a constant rate of glomerular filtration. This animal had its independent renal portal circulation intact which accounts for the relatively low filtration fraction. The renal plasma flow was calculated as the maximal diodrast clearance, and the filtration rate as the creatinine clearance. Renal plasma flow and filtration rate are expressed as milliliters per kilogram per hour and are both plotted on the same logarithmic scale.

ently about half the blood coming to the bullfrog kidney is arterial and half venous. Diodrast is excreted considerably more efficiently than phenol red by the frog. The maximal phenol red clearance obtained with the portal supply intact was 185 ml. per kgm. per hr. at a plasma concentration of 0.5 mgm. per cent (Forster, 14).

SUMMARY

The effects of epinephrine on frog renal hemodynamics as studied by the indirect clearance methods are precisely the same as those noted by direct observation of the functioning kidney. This is interpreted as constituting further

evidence for the reliability of clearance methods in evaluating the various aspects of renal activity.

The administration of small amounts of epinephrine into the circulation results in decreasing the renal blood flow, increasing the filtration fraction and, hence, maintaining a constant rate of glomerular filtration. This effect is obtained by causing constriction of the efferent glomerular arteriole.

Constriction of the afferent arteriole accompanied by decreased renal blood flow, filtration fraction, filtration rate and number of functioning glomeruli results from the administration of massive doses of epinephrine.

In the normal frog, unlike mammals, afferent control of the glomerular circulation plays an important rôle in the regulation of glomerular activity. Variations in the rate of water elimination result primarily from alterations in rate of glomerular filtration and only secondarily from variations in the rate of tubular water reabsorption.

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BLOOD PRESSURE RESPONSES OF DOGS TO VITAMIN A AND VITAMIN D₂¹

L. N. KATZ, S. RODBARD AND J. MEYER

From the Cardiovascular Department, Michael Reese Hospital, Chicago

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Certain findings in the literature appear to indicate that large doses of vitamins A and D have a striking effect on blood pressure. It was felt that this needed reinvestigation.

Vitamin A. The use of vitamin A as an anti-hypertensive agent was first suggested by Govea-Pena and Villaverde (1) who claimed a favorable response of hypertensive patients to large doses of vitamin A. This action was apparently confirmed on dogs with experimental renal hypertension by Wakerlin et al. (2) who reported striking reductions in blood pressure in 3 hypertensive dogs beginning 2 weeks after daily oral administration of 200,000 units of vitamin A in 1 cc. of sesame oil. This reduction persisted for 6 months; the dosage in the last 3 months having been doubled. Sesame oil itself had no such effect on 2 dogs.

We repeated the study using 10 hypertensive and 1 normotensive dog. The hypertensive group included 5 rendered hypertensive by partial occlusion of one or both renal arteries, 3 in which hypertension followed temporary complete occlusion of both renal arteries for about an hour, and 2 in which the hypertension was spontaneous (cf 3). Hypertension in the dogs in the two 1st groups was of 100 to 850 days' duration. A control period of from 33 to 77 days was established in all dogs before instituting vitamin A therapy. Vitamin A dissolved in fish oil² was fed in doses of 400,000 units daily for periods of 45 days. In two of the dogs rendered hypertensive by partial renal artery constriction, a second course of therapy was instituted 56 days after the first. Vitamin A was given for 55 days in the same dosage as before, but dissolved in sesame oil instead of fish oil.³ Blood pressures were recorded with the Hamilton needle manometer as previously described (3). Adequate absorption was tested by determination of the plasma level three weeks after the beginning of treatment according to the method of Carr and Price. A 200-fold increase in the plasma level of vitamin A was found at this time.

There was in no instance a significant change in blood pressure during the first 3 weeks of vitamin A administration. During the last 3 or 4 weeks no significant change was observed in 8 of the 10 dogs used. A drop in diastolic pressure

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² This material, kindly supplied by Abbott Laboratories, contained 100,000 units per cubic centimeter of fish oil.

³ This material was supplied to us by the kindness of Dr. G. Wakerlin. It came from the same supply that he had used for his first study.

occurred in the remaining two. One of these (X-56), a dog with Goldblatt hypertension, which received two courses of treatment, showed drops during this time averaging 26 mm. Hg and 16 mm. Hg below the average diastolic pressure in the control period. These reductions persisted for 25 and 26 days after vitamin A administration was stopped. The second dog, a spontaneously hypertensive animal, showed a drop in diastolic pressure averaging 13 mm. Hg during the second half of the therapeutic period. No toxic symptoms were observed in any of the dogs during or after vitamin A administration.

Our results, therefore, fail to confirm the observation previously reported that vitamin A consistently lowers the blood pressure of hypertensive dogs, but do show that such a response, mild in degree, may be individually encountered. This is in accord with the more recent results of Wakerlin et al. (4) who found no drop in blood pressure in a second group of hypertensive dogs treated with vitamin A.

Whether this occasional depressor action is due to vitamin A itself or to some other material contained in the concentrate, as suggested by the observations of Grollman and Harrison (5), cannot be stated.

An unexpected result obtained in our experiments was the occurrence of a significant rise of the blood pressure after vitamin A medication had been stopped in 7 of the 8 dogs not showing a depressor response during vitamin A treatment. These rises, averaging for the diastolic pressure 13, 16, 17, 21, 23 and 25 mm. Hg above the pre-treatment level in the hypertensive dogs, and 17 mm. Hg in the normotensive dog, began from 14 to 20 days after treatment and persisted for from 27 to 80 days. No rise occurred in the dog whose blood pressure was reduced both times with vitamin A therapy. This post-therapy hypertensive effect would of course be exactly contrary to the expected vaso-depressor effect for which vitamin A therapy was contemplated, and is a contra-indication for the use of vitamin A therapeutically.

It might be argued that in most dogs homeostatic mechanisms powerful enough to counteract the vasodepressor effect are brought about by the ingested material, and that these mechanisms persist unbalanced after therapy is ended. Whether or not this view is valid, does not alter the fact that the vitamin A concentrates used do not lower blood pressure in experimental hypertension except on occasion and then only to a slight degree. This latter possibility is offset by the post-treatment vasopressor reaction which occurs when vasodepression fails to occur during the therapy.

Vitamin D₂. Appelrot (6) first reported that feeding dogs for periods of 15 to 25 days with large quantities of vitamin D (vigantol, 70 to 90 micrograms/kilo body weight/day) produced a hypertension in normotensive dogs. This result was confirmed by Handovsky (7) and Goormaghtigh and Handovsky (8), using oral vitamin D₂ in dogs. Hypertension was reported to begin in a few days when doses of about 100 to 700 micrograms per kilo of body weight per day were used. However, when subacute lethal doses were used (13 to 20 mgm. per kilo body weight per day), hypotension resulted. Reed et al. (9) recently repeated these studies and were unable to obtain any elevation of blood pressure in rats following

oral administration of vitamin D₂ in doses sufficiently large to induce severe reactions such as loss of weight. They state further that they have not been able to produce hypertension in dogs or man with various forms of vitamin D.

In this study we used 10 dogs, 5 dogs with experimental renal hypertension, 3 dogs with spontaneous hypertension and 2 normotensive dogs. In the spontaneously hypertensive dogs, vitamin D₂⁴ dissolved in propylene glycol was administered for 31 days subcutaneously in doses of 40,000 units U.S.P. per day, in the other dogs vitamin D₂⁴ by mouth, 1 mgm. per day, was used (400,000 units U.S.P. per day).

In all but three dogs no significant blood pressure change was observed during or after vitamin D administration. In the three exceptions, significant blood pressure elevation was encountered. In the normotensive dog (Y-39) in which the carotid sinus had previously been denervated, a rise of blood pressure averaging 24 and 16 mm. Hg respectively for systolic and diastolic pressure was noted for the first fifteen days of vitamin D administration, which brought the diastolic pressure up to the lower limits of hypertension. This rise disappeared during the later period of vitamin D medication.

In one of the spontaneously hypertensive dogs (Y-231) the rise in pressure was most marked in the first fifteen days following termination of vitamin D administration. At this time the systolic/diastolic pressures had risen on the average 19/18 mm. Hg above the average control level.

The greatest and most persistent rise occurred in one of the renal hypertensive dogs (Y-84). It amounted on the average to 26/27 mm. Hg during the period of vitamin D administration and was even higher than this during the 15 days after vitamin D administration, amounting to 27/35 mm. Hg above the average control level.

It would appear, therefore, that vitamin D does not usually have a pressor effect in dogs. On occasion, however, it may produce a moderate blood pressure rise, even in animals already spontaneously hypertensive, or rendered so by partial renal artery occlusion. Such individual variation as occurs with vitamin D we have found to be the case with another steroid, desoxycorticosterone acetate (10), except that the pressor effect with the latter is more pronounced and occurs more consistently. The variability in response which we obtained with vitamin D₂ may account for the apparently contradictory reports in the literature. It would, however, be erroneous to leave the impression that vitamin D₂ is a consistent and powerful vasopressor substance. Our results suggest rather that it has this tendency, but only to a slight degree.

SUMMARY

1. We cannot confirm the observations that:
 - a. Vitamin A in large quantities lowers the blood pressure of hypertensive dogs.
 - b. Vitamin D₂ in large quantities raises the blood pressure in normotensive dogs.

⁴ Supplied through the courtesy of Winthrop and Company.

2. However, we have found that on occasion a dog will show:

a. A slight lowering of an elevated blood pressure with vitamin A concentrate in fish oil or sesame oil, and

b. A moderate rise in blood pressure with vitamin D₂.

3. Attention is drawn to the frequent occurrence of a moderate rise in blood pressure of fairly long duration which occurs after a latent period following cessation of vitamin A concentrate therapy.

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THE TRANSFER OF ANDROGENS IN PARABIOTIC RATS¹

ELVA G. SHIPLEY, ROLAND K. MEYER AND CLYDE BIDDULPH

From the Department of Zoology, University of Wisconsin, Madison

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The transfer of hormones in parabiotic rats had been reported by Martins (1), who found that the estrogen produced by the stimulated ovaries of a normal rat in parabiotic union (Coelioanastomosis) with an ovariectomized female was transferred to the latter. Hill (2, 3) confirmed this, using pairs in which the body cavities were not connected. He also presented evidence which indicated that there was a transfer of the corpus luteum hormone from a pregnant rat to its non-pregnant partner. Kallas (4, 5) observed a precocious development of the ovaries in normal immature female rats in parabiosis with castrates, which he attributed to the passage of gonadotropic hormone from the castrated to the normal animal. Biddulph, Meyer and Gumbreck (6) found that relatively large quantities of estradiol or diethylstilbestrol must be injected into one partner of castrated female-female parabiotic rats before detectable amounts cross to the uninjected parabiont. In view of these experimental data it became of interest to determine the amount of androgen necessary to inject into one partner of castrated male-male parabiotic rats to obtain evidence of transfer of the hormone to the other.

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It is seen that there was no stimulation of either seminal vesicles or prostates in the left partner when 15, 25 or 150 gamma of testosterone propionate were injected into the right partner. The accessory organs of the injected partners in

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NO. OF PAIRS	DAILY DOSE (γ)	LEFT-HAND PARTNER		RIGHT-HAND PARTNER*	
		Seminal vesicle weight \pm S.E.	Prostate weight \pm S.E.	Seminal vesicle weight \pm S.E.	Prostate weight \pm S.E.
		mgm.	mgm.	mgm.	mgm.
7		9.1 \pm 0.61	33.5 \pm 2.00	9.5 \pm 1.00	32.5 \pm 4.40
5	15	7.5 \pm 0.90	21.4 \pm 1.64	42.3 \pm 5.83	106.5 \pm 10.84
6	25	7.1 \pm 0.65	27.1 \pm 2.00	75.9 \pm 6.68	157.4 \pm 10.81
6	150	9.3 \pm 0.42	30.0 \pm 2.91	239.3 \pm 26.90	357.6 \pm 13.96
6	300	12.6 \pm 0.78	55.2 \pm 3.42	304.9 \pm 14.30	404.5 \pm 22.60
6	400	13.6 \pm 1.76	45.0 \pm 4.45	379.6 \pm 21.79	491.3 \pm 21.55
6	500	10.1 \pm 0.92	37.1 \pm 3.63	308.8 \pm 37.30	398.3 \pm 28.00
7	750	14.6 \pm 2.00	48.2 \pm 6.40	382.6 \pm 44.30	425.3 \pm 33.10
7	1,000	20.7 \pm 4.20	73.1 \pm 9.24	354.1 \pm 23.18	442.5 \pm 19.15

* Injections were made into the right-hand partner.

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Stimulation of the male accessory organs of the untreated partner was obtained in the parabiotic rats in which the injected partner received 300 gamma or more of testosterone propionate. The mean weight of the seminal vesicles and prostates of the untreated partners of those pairs receiving 300 gamma was approximately the same as that of single animals receiving 6 gamma of testosterone propionate. (See table 2.) This indicates that the equivalent of approximately 6 gamma of testosterone propionate per day crossed from the injected to the uninjected partner under these circumstances. Biddulph, Meyer and Gumbreck (6) using castrated female-female parabiotic rats found that 1 gamma of estradiol or diethylstilbesterol had to be injected into one parabiont before the equivalent of 0.0125 gamma of estradiol or 0.025 gamma of diethylstilbesterol crossed to the uninjected rat. Their data show that a dose equivalent to 80

THE TRANSFER OF ANDROGENS IN PARABIOTIC RATS¹

ELVA G. SHIPLEY, ROLAND K. MEYER AND CLYDE BIDDULPH

From the Department of Zoology, University of Wisconsin, Madison

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times the minimum stimulating dose of estradiol or 40 times the minimum stimulating dose of diethylstilbestrol were required to give evidence of transfer in female-female parabiotic rats. From the data presented in tables 1 and 2 it can be seen that 50 times the minimum stimulating dose of androgen for single castrated male rats was required before there was evidence of transfer from one partner to the other.

We are unable to explain adequately why such large quantities of gonadal hormones must be injected into one parabiont before detectable amounts can be demonstrated in the uninjected partner. Since Kallas (4) and others have shown that castration of a parabiont causes great increase in the size of the gonads of the unoperated partner, it is logical to believe that gonadotropic hormones are more readily transferred across the anastomosed tissue. It is to be emphasized, however, that the source of the hormones is the pituitary gland, that they are water soluble and are secreted directly into the blood stream.

TABLE 2

*Weight of seminal vesicles and prostates of castrated male rats given testosterone propionate**

NUMBER OF ANIMALS	DAILY DOSE (γ)	MEAN SEMINAL VESICLE WEIGHT \pm S.E.	MEAN PROSTATE WEIGHT \pm S.E.
		<i>mgm.</i>	<i>mgm.</i>
7		10.4 \pm 1.50	32.6 \pm 2.56
5	2	10.7 \pm 0.49	33.8 \pm 2.49
5	4	10.3 \pm 1.06	43.1 \pm 2.43
5	6	12.4 \pm 1.12	56.4 \pm 4.71
6	8	24.7 \pm 3.10	71.6 \pm 4.58
6	10	24.4 \pm 1.91	81.8 \pm 4.33

* The mean seminal vesicle and prostate weights of 8 single normal rats were 20.1 \pm 2.57 and 89.6 \pm 9.78 mgm. respectively.

The accessory organs of the injected partners showed an increase in weight with each increase in dosage of the testosterone propionate to and including 400 gamma, beyond which an increase in the quantity given did not produce any consistent increase in weight. The prostates and seminal vesicles of the uninjected partners of those pairs receiving 15, 25 and 150 gamma per day did not show any increase in weight over control pairs; those of pairs given 300, 400, 500 and 750 gamma per day were slightly heavier and the heaviest were obtained in those pairs in which 1000 gamma per day was given. The weights of the seminal vesicles and prostates of the uninjected rats of this latter group were approximately the same as those found in single castrate animals injected with 8 gamma of testosterone propionate per day, or the same as those in normal rats of the same age and weight. This indicates that the equivalent of approximately 8 gamma per day crossed from the injected to the uninjected partner. Hertz and Meyer (8) found that it was necessary to inject 15 gamma of testosterone propionate per day into the castrate male partner of male-female parabiotic rats to prevent ovarian hypertrophy of the normal female. As the data in table 1 show, at least 20 times this quantity of the androgen must be injected into one parabiont before there is any evidence of transfer to the other.

SUMMARY

One partner of castrated male-male parabiotic rats was injected with daily doses of testosterone propionate varying between 15 and 1000 gamma. Evidence of transfer of the androgen from the injected to the uninjected parabiont was found when the daily dose was 300 gamma or more. At this dose level approximately the equivalent of 8 gamma of testosterone propionate crossed to the uninjected parabiont. Seminal vesicles and prostate glands equal in weight to those of single normal rats were obtained in uninjected parabionts when 1000 gamma of testosterone propionate were injected into the other partner.

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EFFECTS OF ANESTHETIC DOSAGE OF PENTOBARBITAL SODIUM ON RENAL FUNCTION AND BLOOD PRESSURE IN DOGS

A. C. CORCORAN AND IRVINE H. PAGE

*From the Lilly Laboratory for Clinical Research, Indianapolis City Hospital,
Indianapolis, Indiana*

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Since many procedures in physiology and experimental surgery require the use of anesthesia, it is important to recognize the deviations from the normal which the anesthetic may produce. Pentobarbital sodium is widely used for experimental anesthesia but, as Mylon, Winternitz and de Sütö-Nagy (1943) point out, certain side effects of the anesthesia may mimic some phases of experimental shock. They note among these untoward effects suppression of renal function and lowering of blood pressure, and consider that, "while there are undoubtedly individual variations in the response to nembutal," i.e., sodium pentobarbital, "on the part of animals, the major factors are the size of the dose and the method of its administration." It was their experience that the use of a 25 mgm. per kilo intravenous dose of the drug was more satisfactory than a dose of 30 mgm. per kilo because it was less toxic. It seems likely that intraperitoneal administration would be still less depressing than intravenous use; the peak of its concentration in the blood would be reached more slowly than with intravenous dosage, while the direct entry of some of the drug into the portal blood would result in its early destruction in the liver. This is the reasoning behind the practice in this laboratory of anesthetizing dogs by intraperitoneal injection of a 2.5 per cent aqueous solution of pentobarbital sodium (Lilly) in a dosage of 30 mgm. per kilo body weight. That the anesthesia thus induced is not commonly associated with evidence of toxic depression is shown by the observations of renal function and arterial pressure which form the basis of this report.

METHODS. The observations of renal function include the measurement of plasma diodrast and inulin clearances, of maximum tubular capacity for diodrast secretion (Tm_D) and rate of urine flow and were made by methods described in detail in previous studies (Corcoran and Page, 1943; Corcoran, Taylor and Page, 1943). The effects of anesthesia are shown by contrasting observations made during anesthesia with those obtained in the same dogs when conscious and when training had accustomed them to the necessary procedures. The record of arterial pressure was obtained from a damped mercury manometer by femoral arterial puncture with a 20 gauge needle when conscious, and by exposure and cannulation of the femoral artery when anesthetized. During anesthesia, the observations begin at about 45 minutes after injection of the anesthetic and continue for from 30 to 50 minutes.

The effect of anesthesia on blood pressure was examined statistically by comparison of 150 observations of femoral arterial pressure in 145 normal dogs

under anesthesia with values derived from 585 observations of femoral arterial pressure in 27 normotensive dogs. To make the data comparable, it is necessary to exclude from the observations of blood pressure in the conscious state those temporary nervous influences which disturb it and which would not operate under anesthesia. The dogs on which the observations were made were for this reason carefully trained to the procedure and in each of these the average of the recorded pressures was calculated. This average was taken as the resting arterial pressure of that animal and the mean and standard deviations obtained from these 27 values were used for comparison with the levels observed in other dogs under anesthesia. It should be noted that the dogs in which the observations were made in the conscious state were not entirely normal, since unilateral nephrectomy and contralateral subcutaneous explantation of the kidney (Page and Corcoran, 1940) had been done before the observations were made. That these operations do not significantly influence arterial pressure is shown by the mean pressure of these dogs, viz., 129 mm. by mercury manometer, a value nearly equal to that of 127 mm. Hg dirotic femoral arterial pressure optically recorded in a group of normal dogs by Hamilton, Pund, Slaughter, Simpson, Colson, Coleman and Bateman (1939). The group is therefore normal as regards arterial pressure. Indeed, any effect of the operations on arterial pressure would probably have been an increase due to altered renal hemodynamics and initiation of mild renal hypertension. In such a case, the difference between normal dogs and those under anesthesia would be still greater than that we describe. The use of data from uninephrectomized dogs with unilateral renal explants is therefore justifiable for the purpose of the comparison.

The observations of arterial pressure are summarized in table 3 by methods suggested by Bradford-Hill (1937). Standard deviation (σ) of group I, the normotensive dogs, was calculated from the average arterial pressures of these 27 dogs as $\sqrt{\frac{\Sigma}{n}} \times \sqrt{\frac{n}{n-1}}$, where Σ is the sum of the squares of differences from the mean and N the number of observations. In group II was calculated $\sqrt{\frac{\Sigma}{n}}$. The standard deviation of the mean was taken as σ/N . The standard error of difference between the means was taken as $\sqrt{\frac{\sigma_I^2}{n_I} + \frac{\sigma_{II}^2}{n_{II}}}$ where the subscript numerals refer to the respective groups of dogs.

RESULTS. I. Effects on Renal Function. a. *Maintenance of function.* That renal function is not impaired by the anesthesia was suggested by values of diodrast and inulin clearance which in 34 of 37 experiments were within the ranges we consider normal for unanesthetized dogs. Proof of this maintenance of function is shown in table 1, in which are summarized observations in 4 dogs in which observations were available during the conscious state. The only deviations observed during anesthesia from values obtained while conscious are (1) in 4 of 8 instances lower rates of urine flow, and (2) in all 8 observations, an increase of arterial pressure.

b. *Occasional failure of renal function.* Evidence of temporary failure of

TABLE 1

Effect of anesthesia on renal function

Absence of effect on pentobarbital anesthesia on renal function. Each observation of clearance or Tm_D recorded is the mean of three periods of urine collection each of about 10 minutes' duration.

DOG NO.	DATE	ANESTHETIZED OR CONSCIOUS	CLEARANCE		FILTRATION FRACTION	URINE VOL.	HEMATOCRIT	ARTERIAL PRESSURE	Tm
			Diodrast	Inulin					
			cc. per min.	cc. per min.		cc. per min.	per cent	mm. Hg	mgm. D-1 per min.
1	8-14	Anes.	172	70	0.41	0.5	44	182	
	8-17	Consc.	165	69	0.42	0.5	39	144	14.4
	12-21	Anes.	170	53	0.31	0.5	50	198	
	12-30	Anes.	165	54	0.33	0.2	40	160	
2	8-21	Consc.	135	46.4	0.34	0.3	38	106	9.6
	8-24	Anes.	111	43.6	0.39	0.5	41	170	9.9
	8-27	Consc.	107	35.5	0.33	0.6	36	120	9.7
	9-15	Anes.	103	35.6	0.33	0.1	36	155	
	10-6	Consc.	119	39	0.33	0.3	39	101	
3	6-30	Consc.	286	78	0.27	0.4	42	128	16.5
	7-6	Consc.	238	76	0.32	0.6	44	129	16.9
	12-6	Anes.	248	81	0.32	0.2	56	158	17.2
4	6-18	Consc.	259	75	0.29	0.5	48	128	16.4
	7-1	Consc.	173	61	0.35	0.8	50	142	17.5
	5-31	Anes.	189	67	0.36	0.3	43	150	16.9
	6-7	Anes.	221	62	0.28	0.5	41	152	18.9

TABLE 2

Depression of renal function during anesthesia

Observations in 2 dogs showing failure of renal function under pentobarbital anesthesia; in one case (a) observations under anesthesia are averaged from 3 periods of urine collection and compared with similar averages from the conscious state; in the other (b) progressive failure of function is shown in succeeding 10 minute periods.

(a) Dog 5

DATE	ANESTHETIZED OR CONSCIOUS	CLEARANCE		FILTRATION FRACTION	URINE VOLUME	HEMATOCRIT	ARTERIAL PRESSURE
		Diodrast	Inulin				
		cc. per min.	cc. per min.		cc. per min.	per cent	mm. Hg
2-26	Conscious	210	61.8	0.29	0.7	44	134
3-8	Conscious	231	64.3	0.28	0.5	42	128
4-27	Anesthetized	47	not done		0.1	51	176

(b) Dog 6

PERIOD OF URINE COLLECTION	DURATION OF COLLECTION						
	minutes						
1	10.5	308	72.5	0.23	0.2	42	124
2	10.0	210	48.8	0.24	0.15		118
3	10.75	98	26.8	0.27	0.1		116

renal function was obtained in 3 dogs of 37 on which observations were made under anesthesia. In one of these (no. 5) observations had been made during the conscious state and the contrast with the effect of anesthesia is shown in table 2a; in another (no. 6) the onset of renal failure under anesthesia is shown in succeeding periods of urine collection (table 2b).

II. *Effect of Arterial Pressure.* In the nine experiments shown in table 1 and table 2a the arterial pressure is consistently greater under anesthesia. This characteristic of pentobarbital anesthesia is not generally recognized. For this reason the data from a large series of experiments were submitted to the analysis described above and summarized in table 3.

TABLE 3

Summary of observations of blood pressure and calculations from (group I) conscious trained dogs and (group II) normal dogs under pentobarbital anesthesia

	GROUP I CONSCIOUS DOGS	GROUP II ANESTHETIZED DOGS
Number of observations.....	585	150
Number of animals.....	27	145
Mean level of arterial pressure, mm. Hg.....	129	146.7
Standard deviation, mm. Hg.....	± 4.8	± 21.1
Standard deviation of mean, mm. Hg.....	± 0.9	± 1.7
Standard error of difference.....	0.99	
Difference of means.....	17.95	
Standard error of difference.....		

DISCUSSION. Our observations show that renal function is usually maintained at normal levels during pentobarbital anesthesia and that blood pressure is usually increased. But it must be realized that these conclusions apply only to conditions of dosage and mode of administration similar to those used in these experiments. Indeed, the occasional failure of renal function in this group suggests that even with the procedure in use we are still on the threshold of toxicity.

The maintenance during anesthesia of normal values of plasma diodrast and inulin clearances and of maximum capacity to excrete diodrast by secretion indicate respectively that renal plasma flow, glomerular filtration rate and one of the tubular functions of the kidneys are unaltered (Smith, 1937; Smith, Goldring and Chasis, 1938). We have shown that the proportion of diodrast extracted from plasma by the kidney is not depressed during anesthesia (Corcoran and Page, 1943; Corcoran, Taylor and Page, 1943) and it follows from this and the maintenance of clearance that the rate of renal blood flow is not altered. But it cannot be concluded that renal circulation is entirely unchanged, for the absence of change of renal blood flow during the increased blood pressure indicates that renal resistance must have been increased. Filtration fraction, i.e., the ratio of inulin to diodrast clearance, is not affected by anesthesia, a fact which suggests that the increased renal resistance is not

associated with a change in the head of pressure within the glomerular capillaries, for a change in filtration pressure would alter the volume of filtrate formed per unit volume of plasma flow through the glomeruli. The site of increased resistance is therefore probably the afferent arterioles, constriction of which would at once maintain renal blood flow and intraglomerular pressure at normal levels during an increase of arterial pressure. This activity of the afferent arterioles is probably an expression of their participation in the general tendency of the renal circulation to maintain itself unchanged during variations of arterial pressure (reviewed by Smith, 1939). That the vasoconstriction is more likely the result than the cause of the increase of arterial pressure is suggested by its locus in the afferent arterioles, for most types of peripheral vasoconstriction which increase arterial pressure are associated with an increase of filtration fraction and evidence of efferent arteriolar constriction, e.g., psychogenic constriction, adrenin (Smith, 1939) renin, angiotonin (Corcoran and Page, 1939, 1940). The homeostasis of renal circulation during the increase of arterial pressure caused by pentobarbital anesthesia thus resembles the renal state during the cardiac hypertension released by injection of atropine and infusion of pitressin (Corcoran and Page, 1939).

The mechanism of the renal failure which occasionally develops under anesthesia is not clear. Our observations suggest that renal plasma flow and glomerular filtration rate are concurrently depressed, while the oliguria, sometimes present when these functions are not altered, is increased. The failure is not the result of arterial hypotension, for it occurred in one animal (no. 5) whose arterial pressure was increased by anesthesia, while in another clearance fell by two-thirds during an 8 mm. Hg decrease of arterial pressure. Since renal plasma flow is decreased, the cause may lie in renal vasoconstriction similar to that observed by Haury, Gruber and Gruber (1939) during intravenous injection of thiobarbiturate. This explanation must remain speculative until simultaneous observations of total renal blood flow and diodrast clearance have confirmed it. Alternatives suggest themselves such as a breakdown of tubular barriers due to concentration of the anesthetic in tubular fluid.

The increase of arterial pressure observed in the 9 experiments of tables 1 and 2 is confirmed as a general reaction to pentobarbital anesthesia by the data of table 3. The differences of mean level of pressure between the conscious and anesthetized dogs may be considered statistically convincing, since this value divided by the standard error of the differences greatly exceeds the value of 3 usually accepted as the test of significance. The increase in pressure may be attributed to sympathetic excitation similar to that which occurs in other types of anesthesia (reviewed by Smith, 1939). Whatever its cause, the fact that it occurs should revise upwards the "normal" levels of arterial pressure to be expected in dogs given pentobarbital sodium as an anesthetic. The level of pressure is a widely accepted indicator of the general condition of anesthetized animals and it is therefore important to realize that, under pentobarbital, levels of 110 or even 120 mm. Hg measured by mercury manometer may be indications of toxic depression. Since renal failure may occur without a decrease of

arterial pressure, it may be that observation of urine flow will serve as an additional and possibly more delicate safeguard in experiments which the depressant effects of anesthesia might disturb.

SUMMARY

Anesthesia induced in dogs by intraperitoneal administration of 30 mgm. per kilo body weight of pentobarbital sodium does not usually impair renal function, for diodrast clearance and tubular secretory capacity as well as inulin clearance may be unaltered as compared with values observed in the resting conscious state. The maintenance unaltered of effective renal plasma flow (plasma diodrast clearance) and filtration fraction during the increase of arterial pressure induced by anesthesia indicates that the afferent arterioles have moderately constricted, in response, not to the anesthesia, but to the hypertension it causes. When renal failure occurs, it is associated with marked oliguria and with concurrent depression of diodrast and inulin clearances independently of changes in arterial pressure. Attention is drawn to the increase of arterial pressure commonly present during pentobarbital anesthesia in dogs. Levels of 110 to 120 mm. Hg which might be accepted as normal in conscious dogs may express toxic depression under pentobarbital anesthesia. It is suggested that the onset of severe oliguria, indicating as it does the onset of renal failure, may provide a more delicate index of the toxic effects of the anesthetic than does a decrease of arterial pressure.

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THE EFFECT OF VITAMIN A ON SOME RENAL FUNCTIONS OF THE DOG

R. J. BING¹

From the Department of Physiology, New York University College of Medicine

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A series of papers have been published in recent years indicating that the feeding of large doses of vitamin A increases renal function in the dog (1) and man (2). Furthermore, reports describing a fall in blood pressure after administration of vitamin A in hypertensive subjects (3) indicate that a possible correlation might exist between the renal action of this substance and the mechanism responsible for its hypotensive effect. The experiments reported in this paper, carried out over a period of two years, were designed to investigate the effect of various doses of vitamin A on the filtration rate, effective renal plasma flow and tubular excretory mass of normal dogs, maintained on a basal diet.

METHODS. All experiments were performed on a total of seven unanesthetized female dogs. The animals were maintained during the whole period of observation on a daily diet consisting of 100 grams crackermeal, 30 grams meat powder,² 30 grams skimmed milk, 3 grams salt mixture and 5000 units of vitamin A.³ Diodrast clearances (4) were used in six, and p-aminohippuric acid clearances (5) in five experiments to measure the effective renal plasma flow. The maximal tubular capacity for the excretion of diodrast (6) was determined in six dogs, p-aminohippuric acid being used in the rest of the animals. Before feeding large doses of vitamin A, normal control values for creatinine, diodrast or p-aminohippuric acid clearances and diodrast or p-aminohippuric acid Tm were established by performing weekly clearances over a period ranging from two to three weeks. Immediately following this control period the feeding of large doses of vitamin A was begun. Fifty thousand units were given to 3 animals, the other 4 receiving 200,000 units, daily. The large doses were continued over a period ranging from two to three months; renal function as described above was determined at bi- or tri-weekly intervals. Following this experimental period, the dosage was reduced to the initial control level of 5,000 units, and the clearance values were again observed for an additional period of three months.

EXPERIMENTAL. The effect of the oral administration of 50,000 units of vitamin A on the renal clearances was inconsistent (table 1). In one animal a slight rise in diodrast Tm values above the control was observed. However, since the diodrast Tm fell while the high dosage was being maintained, the rise was perhaps not caused by the vitamin. In another dog diodrast Tm values remained at the control levels, and in a third animal they rose slightly from 12.6

¹ Supported by a grant from the Josiah Macy Jr. Foundation.

² Valentine Meat Company.

³ E. R. Squibb and Sons.

to 15.8 mgm. diodrast iodine per minute. Since in this last case a further rise was observed 26 days after the high dosage was discontinued, it is probable that

TABLE 1
Effect of 50,000 units of vitamin A

NO. OF DOG	SURFACE AREA	VITAMIN A PER DAY	DAY OF EXPERIMENT	PLASMA CLEARANCES		T _m DIODRAST	FILTRATION FRACTION
				Creatinine	Diodrast		
	sq. m.	units		cc./min.	cc./min.	mgm. iodine per min.	
3	0.691	5,000	5-22-42	78.8	271.0	21.1	0.290
			7-24-42	79.9	206.7	20.5	0.385
		50,000	8-14-42	63.6	194.5	22.17	0.326
			9- 5-42	70.2	214.8	20.2	0.328
		5,000	9-19-42	74.3	209.8	18.95	0.354
			10-10-42	76.3	268.8	19.20	0.284
		5,000	4-17-42	82.0	242.0		0.290
			4-20-42			12.1	
5	0.775	5,000	5-24-42	44.6	162.0	15.8	0.295
			6- 9-42	73.5	205.0	15.8	0.295
			8- 3-42	89.1	214.8	14.5	0.350
		50,000	8-12-42	54.6	160.0	14.7	0.341
			9- 8-42	71.4	213.0	17.5	0.335
			9-15-42	70.8	140.0	15.8	0.372
		5,000	9-30-42	79.4	220.0	19.0	0.360
			10-27-42	77.1	274.0	18.7	0.281
			12-29-42	79.9	306.0		0.260
			1-23-42	37.6	114.0		0.329
			1-28-42	34.8	111.0	9.5	0.314
			2- 4-42	36.8		7.8	
		50,000	2-12-42	38.5	145.0	9.6	0.266
			2-20-42	42.4	145.0	11.6	0.292
			2-26-42	50.0	148.0		0.338
			3- 4-42	44.6	158.0	10.1	0.282
			3-18-42	32.3	161.0		0.201
			3-20-42	43.4	123.0	13.4	0.353
			3-25-42	56.0	175.0		0.320
6	0.450	5,000	3-27-42	32.5		7.70	
			4- 8-42	43.4	152.0		0.285
			6-16-42	49.0			
		50,000	6-18-42	51.6	132.0	9.7	0.391

the increase in the T_m value was either the result of normal variation or the response to threshold dosages. The data obtained on the filtration rate and

TABLE 2
Effect of 200,000 units of vitamin A

NO. OF DOG	SURFACE AREA	VITAMIN A PER DAY	DATE OF EXPERIMENT	PLASMA CLEARANCES			Tm		FILTRA- TION FRACTION
				Creatine	PAH	Diodrast	PAH	Diodrast	
	sq. in.	units		cc./min.	cc./min.	cc./min.	cc./min.	mgm. iodine per min.	
1	0.683	5,000	11-24-42	62.5	184.0		16.5		0.340
			11-30-42	46.1	141.8		16.2		0.325
		200,000	12-21-42	61.0	157.5		17.9		0.387
			2-11-43				19.0		
			3-17-43	66.0	188.2		28.3		0.350
		5,000	4- 5-43	72.4	216.0		19.5		0.336
			5-12-43	77.4	226.0				0.342
			5-21-43	44.3	116.8				0.379
			5-24-43	66.5	208.0				0.319
2	0.715	5,000	11-24-42	52.2	223.5		10.4		0.260
			12- 1-42	59.8	175.7		12.0		0.340
		200,000	1- 6-43	47.6	158.9		17.9		0.299
			3-22-43	68.3	247.5		20.5		0.276
		5,000	4-24-43	47.7	239.9		20.6		0.199
			5-10-43	49.6	164.0		20.2		0.210
			5-26-43	55.3	257.0		16.2		0.216
			6- 2-43	39.8			14.7		
3	0.691	5,000	9- 5-42	70.2	214.8		20.2		0.327
			9-19-42	74.3	209.8		19.0		0.354
		200,000	10-13-42	76.9	219.0		24.6		0.351
			11- 9-42	83.9	279.0		29.7		0.306
			12- 3-42	85.0	283		28.5		0.300
		5,000	1- 8-43	92.8	297.0		29.5		0.312
			1-30-43	76.6	226.0		20.4		0.339
4	0.765	5,000	9-11-42	66.5		214.0		18.9	0.311
			9-22-43	66.1		199.0		18.1	0.321
		200,000	10-16-42	77.0		212.0		21.9	0.363
			11-17-42	75.5		233.0		25.6	0.324
		5,000	12-15-42	62.7		221.0		24.1	0.284
			1-11-43	51.2		174.3		22.1	0.293

the effective renal plasma flow were equally inconsistent showing either no change or fall during the experimental period (table 1). In one instance the creatinine and the p-aminohippuric acid clearances rose following the discon-

tinuation of the high vitamin A dosage (table 1). It was evident, therefore, that the feeding of 50,000 units of vitamin A had no significant effect upon the renal functions observed. This conclusion was further confirmed by statistical treatment of these data. Table 3 shows the maximal range for the normal variation obtained statistically from 32 observations, each consisting of two collection periods. Twelve of these observations were obtained with p-aminohippuric acid, the rest with diodrast. The highest values obtained for each individual animal during the ingestion of 50,000 units lie below the maximal range.

The effect of the daily addition of 200,000 units of vitamin A to the standard diet of 4 dogs resulted in a significant rise in the maximal tubular capacity for the excretion of diodrast or p-aminohippuric acid, the increase averaging from 50 to 100 per cent of the control value (table 2). Figure 1 representing a typical experiment shows that the administration of 200,000 units was effective within

TABLE 3
Deviation of experimental Tm data from maximal normal range

NO. OF DOG	NO. OF UNITS VITAMIN A GIVEN	SURFACE AREA <i>sq. in.</i>	AVERAGE OF HIGHEST EXPERIMENTAL POINTS		MAXIMUM RANGE FROM CONTROL DATA	
			TmD	TmPAH	Diodrast	PAH
1	200,000	0.683		41.4		28.4
2		0.715		28.7		28.4
3		0.691		43.0		28.4
4		0.765	33.4		35.2	
5	50,000	0.775	20.4		35.2	
3		0.691	32.7		35.2	
6		0.450	29.8		35.2	

a period of three weeks. Similar results were obtained in 2 other dogs. In the fourth animal the rise in Tm did not appear until after five weeks. When these data are treated statistically in the manner described above it is seen that the highest Tm figures per square meter of body surface, as obtained on three dogs, exceeded the maximal range of the control data, indicating a significant increase in the renal excretory mass (table 3). In the fourth animal the highest Tm figure lay within the normal variation. Upon individual treatment of the data obtained on this animal, however, it can be seen that a rise in the Tm value above its control data resulted from the application of the vitamin (table 2). A rise was observed in the filtration rate and the effective renal plasma flow of all but one dog, in which the high vitamin A dosage produced no effect. Since this increase ranged from 5 to 30 per cent of the control figures, it was less than the increase observed for the corresponding Tm values. It seems of special significance that the filtration fraction did not change during the vitamin A hyperemia, an observation confirming the results of Corcoran and Page (2). The effect

of vitamin A differs, therefore, from that produced by pyrogens (7) or yeast adenylic acid (8) in which the filtration fraction falls during the hyperemic period.

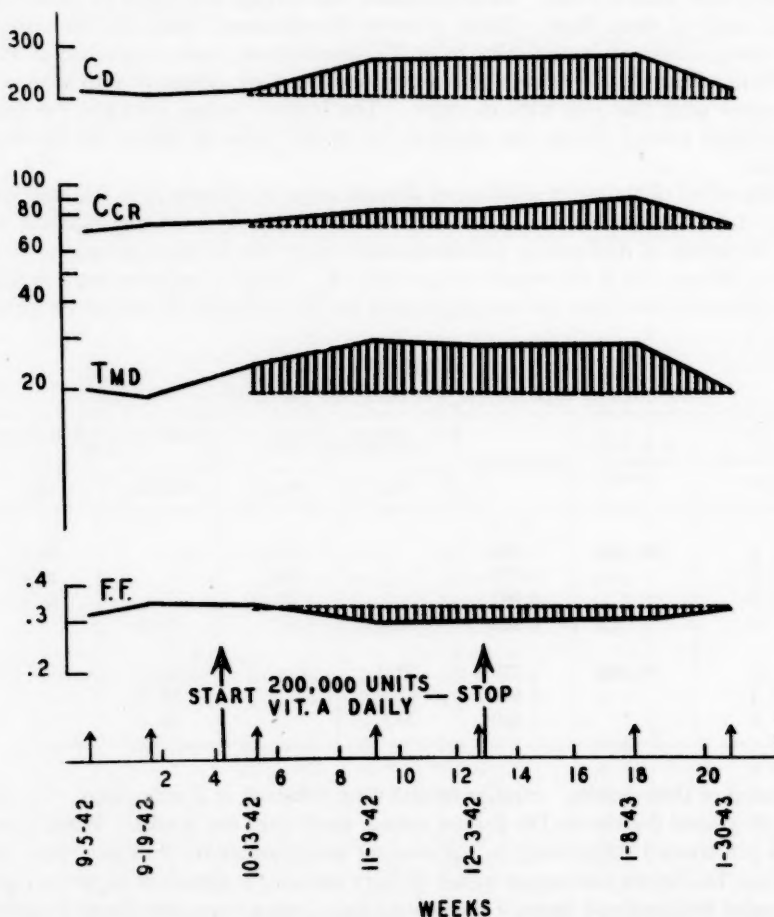


Fig. 1 demonstrates the effect of the daily administration of 200,000 units of vitamin A on the effective renal plasma flow, C_D , the filtration rate, C_{CR} and the maximal tubular excretory capacity for diodrast, T_{MD} .

DISCUSSION. The experiments reported in this paper indicate that the addition of 5,000 as well as 50,000 units of vitamin A has no significant effect upon the glomerular filtration rate, effective renal plasma flow or tubular excretory mass, while the daily administration of 200,000 units causes a significant increase in T_m values in all animals observed. The renal plasma flow and the filtration rate showed a moderate increase in three animals and no change in the fourth.

The rise observed after these large doses is not attributable to correction of avitaminosis, since the dogs had been maintained on a presumptively adequate vitamin intake during the control period. It is also improbable that the rise was caused by free essential fatty acids, since the corn oil used as vehicle for the vitamin contained no fatty acid radicals (9).

From tables 1 and 2 it will be seen that the filtration fraction remained constant throughout the period of hyperemia. This fact indicates that the filtration pressure in the glomerulus remains constant if it is assumed that filtration equilibrium is reached in the glomerular capillaries (10). It is probable, therefore, that the changes in renal blood flow produced by the vitamin A must be mediated by changes in both the afferent and efferent glomerular arterioles. Consequently the mechanism of renal hyperemia described in this paper is similar to that observed to follow the feeding of fish to the harbor seal (11) and of a high protein diet to normal and hypertensive dogs (12).

The effect of large doses of vitamin on the maximal rate of diodrast and p-aminohippuric acid excretion resembles the action of testosterone on the kidney of dog and man (13). In contrast to testosterone, however, vitamin A caused an increase in the effective renal plasma flow and filtration rate in three out of four animals. The rise in the T_m figures may indicate the development of a true renal hypertrophy, an assumption supported by the work of Korenchevsky and others (14) who found that testosterone produces a significant increase in kidney weight. It is possible that the rise in plasma flow and filtration rate observed after the feeding of 200,000 units of vitamin A represents a circulatory adjustment of the kidney to the increased tubular mass of that organ.

SUMMARY

The oral administration of 5,000 and 50,000 units of vitamin A produces no significant change in the glomerular filtration rate, the effective renal plasma flow and the tubular excretory capacity for diodrast or p-aminohippuric acid of normal unanesthetized dogs kept on a standard diet.

The feeding of 200,000 units of the vitamin results in a significant rise in the tubular excretory mass, and a moderate increase in renal plasma flow and filtration rate. No change in filtration fraction occurs during the hyperemia.

The relation of vitamin A to other renotropic substances is discussed.

I wish to express my gratitude to Miss Christine Waples and Mrs. Louise Buchanan for their assistance in performing the experiments.

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tion

THE EFFECT OF ATROPINE AND QUINIDINE SULPHATE ON ATROPHY AND FIBRILLATION IN DENERVATED SKELETAL MUSCLE¹

D. Y. SOLANDT, D. B. DELURY AND JOHN HUNTER

137: *From the Departments of Physiology, Physiological Hygiene and Mathematics,
University of Toronto*

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Fibrillation was first observed by Schiff (10). Langley and Kato (7) ascribed the loss in weight following motor denervation to this activity. Having inhibited fibrillation effectively with quinidine sulphate, and finding little change in atrophy, Solandt and Magladery (11) questioned the overwork hypothesis of Langley (6). Soskin and co-workers (9, 12), using atropine sulphate to decrease fibrillation and physostigmine to increase fibrillation, obtained weight changes in denervated muscle which they interpreted as supporting Langley's hypothesis. The present work was undertaken to obtain new evidence on the relation of atrophy to fibrillation in denervated muscle.

METHODS. One hundred and fifty male albino rats (average weight 220 grams) were used. Denervations were carried out by sciatic nerve section high in the thigh. The choice of the leg to be denervated and the assignment of animals to the treatment to be received were made at random, using Tippett's Random Sampling Numbers (13). Five groups of animals were designated as follows:

- (1) Thirty-seven rats to receive atropine sulphate 38 mgm. daily per 100 grams.
- (2) Thirty-seven rats to receive quinidine sulphate 38 mgm. daily per 100 grams.
- (3) Twenty-six rats to receive atropine sulphate 19 mgm. daily per 100 grams.
- (4) Twenty-six rats to receive quinidine sulphate 19 mgm. daily per 100 grams.
- (5) Twenty-four rats to receive saline.

A larger number of animals was assigned to be given large doses (38 mgm./100 grams) of atropine and quinidine sulphate because of the higher mortality to be expected in these categories. Moderate doses (19 mgm./100 grams) of the drugs were given to other animals as indicated. In each group the drug was administered subcutaneously and the dose volume in all cases was the same. One half of the daily dose was given at 10 a.m. and the other half at 10 p.m. starting the day after denervation. On the 4th, 8th, 12th, 16th and 20th days after denervation 4 animals were selected at random for test and autopsy from each surviving group.

Fibrillation was recorded using a four-stage valve amplifier and a loudspeaker. Paired electrodes consisting of no. 26 hypodermic needles fixed at a distance of 1 cm. apart were used. One pair of electrodes was inserted into the denervated muscles. The placing of the electrodes was done after careful palpation and observation. A second pair of electrodes was inserted in a like manner into the

¹ Aided by a grant from The National Foundation for Infantile Paralysis, Inc.

intact muscles of the opposite limb. A double pole double throw switch permitted a ready comparison of the fibrillary potentials of the denervated muscles with the action potentials of normal tonic activity in the intact muscles. In evaluating this difference the output of the amplifier was led to a loudspeaker and the difference in sound intensity between the denervated and the opposite intact groups of muscles was measured using a sound-level meter (General Radio

TABLE 1

DRUG	DAYS AFTER DENER- VATION	FIBRILLATION (ESTIMATED BY SOUND-LEVEL METHOD AND EXPRESSED AS DENER- VATED MINUS INTACT MUSCLE ACTIVITY)	LEG	BODY WEIGHT		MUSCLE WEIGHT	
				Initial	Final	Dener- vated	Intact
				grams	grams	grams	grams
Large quinidine	4	0	Left	262	263	1.38	1.48
	4	2	Left	257	254	1.23	1.51
	4	0.5	Right	232	249	1.42	1.55
	4	0	Left	220	200	1.18	1.25
Large atropine	4	17.5	Right	217	196	0.94	1.16
	4	10	Left	246	218	1.16	1.20
	4	12	Right	256	216	1.26	1.31
	4	14	Right	200	165	0.85	1.01
Moderate quinidine	4	8.5	Right	198	202	1.19	1.25
	4	7	Left	248	231	1.15	1.21
	4	17.5	Right	180	187	0.86	1.16
	4	16.5	Right	218	230	1.21	1.38
Moderate atropine	4	10	Left	264	231	1.22	1.34
	4	12	Right	200	170	0.90	1.00
	4	7	Left	210	189	1.00	1.03
	4	14.5	Right	192	185	1.00	1.14
Saline	4	12	Right	181	193	0.99	1.17
	4	14	Right	266	285	1.51	1.73
	4	17.5	Left	274	266	1.55	1.75
	4	12.5	Left	180	188	0.98	1.15
Large quinidine	8	1	Right	176	158	0.86	0.93
	8	13	Left	193	168	0.72	0.84
	8	6	Right	183	192	0.60	1.08
	8	8	Left	214	206	0.84	1.16
Large atropine	8	5	Right	265	183	0.91	0.91
	8	7	Right	248	190	0.73	0.89
	8	14	Right	238	166	0.52	0.77
	8	13	Right	180	169	0.65	0.97
Moderate quinidine	8	6.5	Right	186	200	0.87	1.24
	8	9	Left	220	221	1.04	1.42
	8	11	Right	199	230	0.88	1.40
	8	9	Left	240	246	0.96	1.38
Moderate atropine	8	4	Right	178	162	0.67	0.87
	8	9	Left	188	181	0.72	1.00
	8	9	Left	250	235	1.08	1.34
	8	10	Right	195	182	0.75	1.04
Saline	8	6.5	Right	194	207	0.97	1.39
	8	14	Left	274	267	1.07	1.76
	8	15	Left	222	237	1.16	1.66
	8	13	Left	274	243	1.04	1.69

TABLE 1.—Continued

DRUG	DAYS AFTER DENER- VATION	FIBRILLATION (ESTIMATED BY SOUND-LEVEL METHOD AND EXPRESSED AS DENER- VATED MINUS INTACT MUSCLE ACTIVITY)	LEG	BODY WEIGHT		MUSCLE WEIGHT	
				Initial	Final	Dener- vated	Intact
				grams	grams	grams	grams
Large quinidine	12	0	Left	188	176	0.56	1.03
	12	2	Left	194	190	0.76	1.08
	12	1.5	Left	202	182	0.57	0.88
Large atropine	12	5	Left	198	165	0.34	0.70
	12	5	Left	175	150	0.43	0.60
	12	15	Right	199	159	0.41	0.66
	12	14	Left	224	163	0.48	0.60
Moderate quinidine	12	11	Left	233	242	0.41	1.03
	12	3	Right	250	226	0.87	1.30
	12	15	Left	289	300	0.91	1.67
	12	11	Left	255	252	0.87	1.52
Moderate atropine	12	16	Left	204	181	0.57	0.97
	12	13	Right	234	181	0.80	1.10
	12	22	Left	211	180	0.69	0.87
	12	15	Left	214	200	0.84	1.22
Saline	12	4	Right	186	243	0.81	1.61
	12	4	Right	286	297	1.01	1.80
	12	7	Right	245	264	0.97	1.68
	12	6	Left	215	228	0.87	1.60
Large atropine	16	2.5	Right	260	187	0.61	0.98
	16	1	Left	228	160	0.49	0.81
Moderate quinidine	16	0	Left	205	230	0.59	1.30
	16	2.5	Right	180	222	0.66	1.28
	16	2.5	Right	212	193	0.46	1.04
	16	3.5	Right	180	230	0.50	1.31
Moderate atropine	16	5	Right	191	178	0.99	0.99
	16	6.5	Right	237	204	1.11	1.11
	16	5	Left	252	172	0.99	0.99
	16	9	Right	229	180	1.21	1.21
Saline	16	10	Right	212	215	0.74	1.70
	16	7	Right	186	235	0.64	1.43
	16	9	Right	219	245	0.75	1.77
	16	2	Right	240	282	0.69	1.75
Moderate quinidine	20	1	Left	270	254	0.54	1.47
	20	3	Left	260	273	0.62	1.56
	20	2	Right	205	212	0.49	1.13
	20	11	Left	255	254	0.49	1.61
Moderate atropine	20	12	Right	241	215	0.40	1.07
	20	5	Right	211	199	0.57	1.11
	20	8	Left	265	187	0.53	0.99
Saline	20	6	Left	180	274	0.59	1.62
	20	3	Left	210	247	0.67	1.68
	20	8	Right	221	260	0.80	1.79
	20	3	Right	256	288	0.59	1.98

—Type 759A). The sound-level meter served the purpose of an output meter, sufficiently damped to give an integrated reading of the amplifier output. The

use of a suitably damped output meter would have served the same purpose and eliminated the frequency response limitations of the loudspeaker which, however, proved unimportant in the present instance. The records were taken under sufficient ether anesthesia to avoid movement on the part of the animal. Controls indicated that the degree of anesthesia, over the range used, did not measurably affect the fibrillation.

The fibrillary potentials were also photographed using a Matthews oscillograph. Fibrillary activity was estimated by counting action potential spikes. This method yielded results quantitatively similar to those obtained by estimating integrated amplifier output with a sound-level meter.

A visual method of estimating the degree of fibrillation was also attempted. Prior to autopsy, fibrillation on the proximal, middle and distal thirds of the anterior and posterior surfaces of the muscle was observed under reflected light.

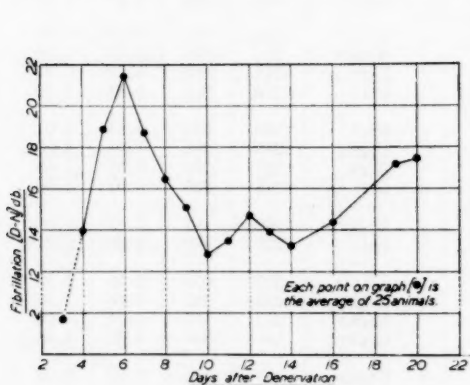


Fig. 1

Fig. 1. Fibrillation in the denervated gastrocnemius-soleus muscle group of the albino rat.

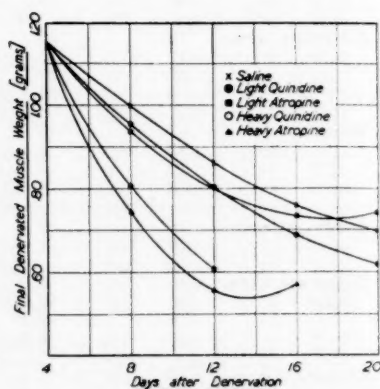


Fig. 2

Fig. 2. Denervated muscle weight for each group of rats adjusted to a fixed value of fibrillation and to the same initial body weight.

Activity was recorded as absent (0), slight (1) or marked (2). Visual methods, although lacking accuracy and objectivity, permitted a crude but useful check on conclusions arrived at by the more precise methods described. On limited trials there was a satisfactory correlation between the results obtained using the three methods for estimating fibrillation.

A series of 25 rats, each with one sciatic nerve cut, was studied, using the sound-level method, to follow the fibrillary activity in animals to which no drug had been administered. The average course of fibrillation for this series is shown in figure 1. Fibrillation was readily detected on the 3rd day after denervation. It increased rapidly to a maximum, generally observed on the 6th day, after which it continued prominent for the duration of the experiment (20 days). The initial increase to the maximum reached on the 6th day was remarkably constant. From the 6th until the 9th day a fairly regular decrease was noted. From the 9th to the

20th day the daily records on individual animals showed some variation from day to day.

In the main series of animals, to which drugs or saline were administered, the variables recorded were: (1) Final weight of the denervated muscle (w). (2) Final weight of the intact muscle (i). (3) Initial body weight (y). (4) Final body weight. (5) Amount of fibrillation (x). (6) Duration of the period of denervation (t). (7) Drug, if any, employed (z).

The experiment was intended primarily to investigate the relationship between (1) and (5). The time variable (6) was introduced simply to provide a wider basis for whatever conclusions might be reached regarding this relationship. The various drug applications (7) were used to produce varying degrees of fibrillation. Initial body weight (3) was recorded for the purpose of establishing control over this variable as a potential source of experimental error. The remaining variables

TABLE 2

REGRESSION COEFFICIENTS		t^*
Symbol	Value	
A	0.0771	
B (Time)	-0.1604	-14.5**
C (Time ²)	0.0315	3.6**
D (Fibrillation)	-0.0031	-1.07
E (Initial Body Weight)	0.2882	6.3**
F (Drug)	0.0555	5.55**

* R. A. Fisher, 1934, Section 29.

** Significant at 1 per cent level.

TABLE 3

Final muscle weight

TREATMENT	REGRESSION COEFFICIENTS	t
Large quinidine	D (Fibrillation)	-0.79
	E (Initial body weight)	0.88
Large atropine	D	-0.73
	E	2.06
Moderate quinidine	D	-0.843
	E	1.596
Moderate atropine	D	0.468
	E	-2.4*
Saline	D	1.359
	E	2.72*

* Significant at 5 per cent level.

(2) and (4) were included to permit an examination of the effect of the drugs when denervation was not present, since any conclusion as to the relationship of (1) and (5) must rest in part on a demonstration that the effects have not been the result solely of the direct action of the drugs.

Two attempts to determine the relationship of atrophy to fibrillation were made. In the first (table 2) a regression equation of form $W = A + Bt + Ct^2 + Dx + Ey + Fz$, where W represents the adjusted value of w , was fitted to show the dependence of w on t , x , y and z (2). A , B , C , D , E and F represent partial regression coefficients. A partial regression coefficient which differs significantly from zero indicates that the corresponding variable exhibits a real relationship with w apart from the effects of the other variables. One which does not differ significantly from zero indicates that the experiment does not give adequate evidence of such a relationship.

A possible objection to this procedure arises from the fact that the variable "drug" is not measurable, and consequently, in order to include it in the analysis,

numbers were assigned arbitrarily to drugs, in such a way that the drug numbers have the same rank as the effect on body weights produced by these drugs. Such a device is not expected to be wholly satisfactory, although it is better than completely neglecting the "drug" factor.

In the second attempt (table 3), this arbitrary element is avoided by fitting a regression equation of form $W = A + Bt + Ct^2 + Dx + Ey$ to the records of each set of rats receiving the same drug. This approach is made possible by the circumstance that fibrillation effects varied considerably within each such set. Differences among these regression equations will presumably indicate differences among the effects of the drugs, but in each of them the fibrillation regression coefficient may be tested as before.

A similar treatment was accorded final intact muscle weight according to the equation $I = A + Bt + Ct^2 + Ey$, where I represents the adjusted value of i , for the purpose of estimating the effect of the drugs when denervation is not present.

In the final evaluation of the effects of body weight (table 4) covariance methods were used (3).

TABLE 4

SOURCE	DEGREES OF FREEDOM	SUM OF SQUARES UNADJUSTED	DEGREES OF FREEDOM	SUM OF SQUARES ADJUSTED FOR INITIAL BODY WEIGHT	DEGREES OF FREEDOM	SUM OF SQUARES ADJUSTED FOR INITIAL AND FINAL BODY WEIGHTS
Time.....	2	0.41467				
Drugs.....	4	0.21957**	4	0.16750**	4	0.04138 n.s.
Error.....	8	0.03933	7	0.02877	6	0.02432

** Significant at 1 per cent level.

n.s. Not significant at 5 per cent level.

RESULTS AND DISCUSSION. Table 1 contains the untreated experimental data, obtained in the manner described, and is self-explanatory. Table 2 shows that the regression coefficient associated with fibrillation alone fails to exhibit significance. We have, therefore, no evidence that fibrillation has any effect on the final weight of the denervated muscle. Duration of denervation, initial body weight, and drug effects all prove to be significant factors contributing to the final weight of the denervated muscle. A similar analysis carried out using visual estimations of fibrillation likewise produced a non-significant fibrillation regression coefficient.

In the regression equations fitted to the observations on each drug group, the coefficients denoting fibrillation effects again proved to be non-significant (table 3). Thus these coefficients, although less precise than those of table 2 (since they are based on smaller numbers of animals), lead to the same conclusion.

The present experiments thus confirm the original conclusion of Solandt and Magladery (11) that fibrillation is not the cause of the atrophy observed in denervated skeletal muscles. Lazere, Thomson and Hines (8) and Weddell, Feinstein and Pattle (14) have presented evidence which supports this conclusion.

The equations for each drug group also demonstrate, through the differences

between them, that the responses to the various drugs were substantially different. The curves of W against t , for fixed values of x and y , are shown in figure 2. These curves indicate the effect of the treatments used on the weight of the denervated muscle. The least final denervated muscle weight was obtained with large doses of atropine. Large doses of quinidine did not depress the final weight of the denervated muscle quite so far and moderate doses of atropine, quinidine and saline were progressively better in that order. The upward trend in final muscle weight shown by the rats receiving large doses of atropine on the 16th day and those receiving moderate doses of atropine on the 20th day is probably due, in

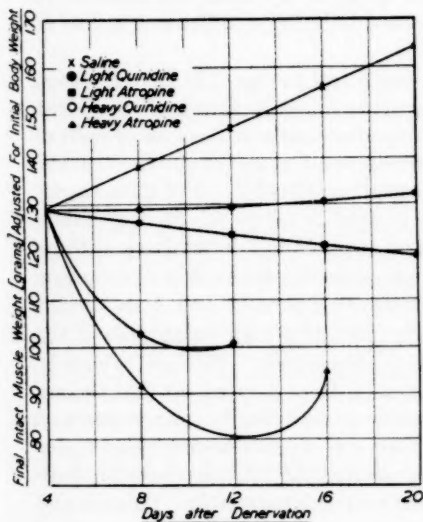


Fig. 3

Fig. 3. Final intact muscle weight for each group of rats adjusted to the same initial body weight.

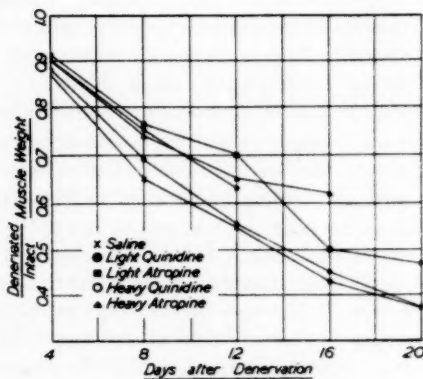


Fig. 4

Fig. 4. Showing the inversion of true results obtained by plotting the ratio denervated over intact final muscle weight in an experiment involving the use of procedures affecting both muscles.

part at least, to the circumstance that unusually heavy rats contributed these points on the graph. The first degree adjustment performed in these cases might not be entirely adequate. Even if this late weight recovery is a real effect, possibly due to accommodation to the drug, it is one shared by both intact and denervated muscles. These experiments give no support to the view that atropine sulphate has a selective beneficial effect on denervated muscle.

Characteristic changes in body weight were seen with each treatment used. Rats receiving saline demonstrated an increase, as was to be expected in growing animals, and this increase was also in evidence when moderate doses of quinidine were administered. Large doses of quinidine and moderate doses of atropine

resulted in some loss while large doses of atropine resulted in a profound loss of body weight. Therefore the question may be raised as to whether, for example, the severe atrophy of denervated muscle exhibited by rats receiving large doses of atropine was the result of action by the drug on the denervated muscle, or if this weight loss simply reflected a general loss of body weight. Some light was thrown on this question by the curves of final intact muscle weight plotted against time, adjusted for varying initial body weight according to the equation $I = A + Bt + Ct^2 + Ey$. These curves (fig. 3) demonstrate that at least a large part of the weight loss of the denervated muscle is also exhibited by the intact muscle. Consequently much if not all of the difference, between one drug and another, in weight loss of the denervated muscle must be attributed to difference in loss of body weight produced by those drugs.

Levine, Goodfriend and Soskin (9) and Soskin and Levine (12) have reported phenomenal weight retention in the denervated muscles of animals receiving large doses of atropine sulphate. In these experiments the ratio of the weight of the denervated muscle to the weight of the corresponding intact muscle from the opposite limb is used as a measure of the existing atrophy. The data of our present experiments, using this ratio, are plotted in figure 4. Here it would appear that moderate doses of quinidine and atropine and large doses of these same drugs, in this order, are progressively more effective than saline in retarding weight loss in the denervated muscle. However, figures 2 and 3 show that animals yielding high values of this ratio are exhibiting high weight loss of the intact muscle, not low weight loss of the denervated muscle. Fischer (1) corroborates this point. Hines, Thomson and Lazere (4) have pointed out that the use of the intact member as a control in drug studies is inadvisable. Knowlton and Hines (5) likewise showed that in cases where the animals undergo significant changes in body weight after denervation the assumption that the control muscle represents the initial weight of the denervated muscle is untenable. Solandt and Magladery (11) used this ratio to indicate the extent of atrophy. The method did not lead to erroneous conclusions in this case because the dose of the drug used was not large and the body weight loss slight as compared to the loss exhibited in the present experiments.

Loss of body weight may account for the whole of the differences between drug effects. This possibility may be tested by adjusting these differences for variations in both initial and final body weight using covariance methods. In order to avoid gaps in the data, observations made after the 12th day were deleted. Table 4 shows the sums of squares of deviations from the mean, of the average weights of denervated muscle for the 5 treatments used, unadjusted, adjusted for initial body weight, and adjusted for both initial and final body weights. When the differences in initial body weight are accounted for the treatment differences are still significant, but when both initial and final body weights are taken into account the treatment differences are no longer significant. We conclude that whatever differences have been observed among the weights of the denervated muscles under different drug applications can be accounted for on the basis of loss in body weight. It appears, therefore, that the drugs have had no demonstrable specific effect on the weight of the denervated muscles.

SUMMARY

1. The atrophy of denervated skeletal muscle is not produced by fibrillation nor is it significantly affected by fibrillary activity.

2. Large, repeated doses of atropine or quinidine sulphate give rise to a reduction in animal body weight and this includes weight-loss on the part of both intact and denervated skeletal muscles.

3. Neither atropine nor quinidine sulphate exerts any specific effect on the weight of denervated skeletal muscle.

The authors appreciate the interest which Prof. C. H. Best has taken in this work.

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THE EFFECT OF TEMPERATURE OF THE BLOOD ON THE HEART RATE¹

ERNEST K. LANDSTEINER AND MORRIS HAYES

From the Departments of Pathology of The Children's Hospital and Harvard Medical School

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The effect of variations in the temperature of the blood flowing through the heart in the heart-lung preparation was studied in the dog by Knowlton and Starling in 1912 (1). Under the conditions of that experiment, the temperature of the venous blood of the heart was the only factor affecting the pulse rate; the rate was unaffected by changes in the arterial or venous pressure. When the temperature of the blood lay between 26.5°C. and 43.0°C., the pulse varied from 63 to 165, and bore an approximately linear relation to the temperature. The heart rate decreased when the temperature of the blood exceeded 43.0°C. Kisch, in 1923 (2), by means of an arterio-venous anastomosis through a paraffin-lined cannula kept in a water-bath between 50°C. and 60°C., noted a rise of 20 to 40 beats in the pulse of rabbits for each 0.5°C. rise in the temperature recorded in the mediastinum. Similar experiments in dogs showed a ten-beat increment per 0.5°C. rise in the temperature recorded in the right auricle. The effect of cooling the blood was not investigated. Section of the vagus nerves or the sympathetics did not abolish the tachycardia produced under these experimental conditions.

In the course of studies unrelated to the problem discussed here there were observed alterations in the heart rate which appeared to be associated with variations in the temperature of even small amounts of fluid injected into the external jugular vein. The paucity of data concerning the effect of temperature of the blood on the heart rate of the intact animal led us to investigate this problem further. Records were made, therefore, of the alterations in the heart rate produced by injection into the external jugular vein of the intact animal of fluid, the temperature of which was varied from 22.0°C. to 57.0°C.

METHODS. Five rabbits and six cats, all healthy adult animals weighing between 3 and 3.5 kgm., were used. Pulse and blood pressure were measured by means of a glass cannula in the left carotid artery connected to a Huerthle membrane manometer and recorded kymographically. Venous pressure was measured with the aid of a cannula in the right external jugular vein, pointing cephalad, and recording in similar fashion. Another cannula was placed lower in the same vein, pointing toward the heart, for the purpose of infusion. Both membrane manometers were calibrated in millimeters mercury. Respirations were recorded from the side arm of the tracheal cannula by means of a Marey tambour. Preparation for the experiment included intravenous injections of heparin, 150 units per kilogram. Three rabbits were anesthetized with sodium pentobarbital (30-35 mgm. per kgm. given intraperitoneally) and two with

¹ This investigation has been aided by a grant from the Josiah Macy Jr. Foundation.

novocaine infiltration of the skin and subcutaneous tissues of the neck. Three cats received sodium pentobarbital in the same dosage and by the same route; three were given light intratracheal ether anesthesia with no premedication.

Physiologic solution of sodium chloride was employed in all experiments. Infusions were given from a 250 cc. infusion flask, suspended approximately

TABLE 1

Pulse variations from the normal caused by infusions of normal saline at different temperatures

	TEMPERATURE OF SALINE INFUSED	ANIMAL—RABBIT	ANIMAL—CAT
	°C.		
Total 69	22-26	-60 -80	-40 -50 -40
	27-31	-30 -40 -40 -50 -50 -60 -60 -60 -60 -60 -100 -100	-10 -10 -10 -20 -20 -20 -20 -20 -20 -20 -30 -30 -30 -30 -30 -40 -40 -40 -40 -40 -50 -60 -60
	32-36	-30 -30 -30 -30 -40 -40 -40 -40 -50 -50 -50 -60 -60 -60	-10 -10 -10 -10 -20 -20 -20 -20 -20 -20 -30 -30 -30 -30
Total 39	37-40	-10 -10 000 000 000 000 000 +5 +5	-5 -10 -10 000 000 000 000 +10 +10 +10 +10 +10
Total 31	41-45	+10 +10 +10 +20 +20 +20 +30	000 +5 +10 +10 +20 +20 +20
	46-50	+20 +40 +50	+10 +20 +20 +40
	50-51	+40 +50 +50 +60	+20 +20 +30 +40

one meter above the level of the cannula and connected to it by means of transparent rubber tubing. A thermometer was held in place in the flask at the point of outflow of the fluid. The tubing connecting the flask and the cannula held about 15 cc. of fluid. Because of the cooling which occurred in this rubber tube (from 40°C. to 33°C. in 1-2 min.) it was necessary to empty it immediately

prior to each infusion. This was accomplished by interposing a three-way stopcock in the system just above the cannula, and allowing 30 cc. of fluid to escape from the sidearm.

Since the fluid beyond the stopcock could not be drained, 2 cc. of saline solution were allowed to run slowly into the jugular vein a few seconds before every test infusion, to exclude the effect of fluid of unknown temperature. The infusions were given at rates which varied from 150 to 200 cc. per minute and from 2 to 25 cc. in amount. Precautions were taken to prevent overloading of the vascular system in the course of the experiments. A total of 69 cool (22°C. to 36°C.) and 31 warm (43°C. to 57°C.) infusions were given. These were controlled by 39 infusions of fluid at "body" temperature (within 2.0°C. above or below the rectal temperature). In almost all experiments observations were made both before and after vagotomy.

The results of these experiments may be listed:

1. Infusions of saline solution at "body" temperature produced no change in the heart rate.
2. Infusions of saline solution at a temperature below that of the body produced a bradycardia in all experiments. This, depending upon the temperature of the infusion, varied from 20 to 100 beats.
3. Infusions of saline solution at a temperature above that of the body caused a tachycardia of from 10 to 90 beats.
4. These effects occurred equally well both before and after bilateral cervical vagotomy.
5. A number of infusions of cool saline (30°C.-35°C.) given into the femoral vein and into the pulmonary artery produced no alteration in the heart rate.
6. In each case the change of pulse rate began 2 to 3 seconds after the beginning of the infusion and lasted for several seconds after all the fluid had been injected. No consistent or important changes in general blood pressure were caused by the infusions. The venous pressure always rose moderately during the administration of the fluid.

DISCUSSION. The generalization may be made that within the limits of the temperatures employed in these studies the lower the temperature of the saline solution the greater the degree of bradycardia, and the higher the temperature the greater the degree of the tachycardia.

The fact that this effect occurs after vagotomy and (as has been shown by Kisch with heat) after sympathectomy indicates probably that local conditions—possibly direct action on the Keith Flack node—are of importance.

The absence of alterations in the pulse rate when saline solution was introduced into the femoral vein and pulmonary artery suggests that in these instances mixing and warming of the fluid injected take place in its transit to the right heart.

SUMMARY

In the intact cat and rabbit, the injection of cool physiologic saline solution (22°C.-36°C.) into the external jugular vein results in a bradycardia and con-

versely the injection of warm saline solution ($43^{\circ}\text{C}.$ - $57^{\circ}\text{C}.$) into the external jugular vein results in a tachycardia. This effect is not altered by bilateral cervical section of the vagus nerves, nor by the action of the several types of anesthetic agents employed.

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THE EFFECT OF THE ADMINISTRATION OF ESTROGEN ON THE MECHANISM OF ASCORBIC ACID EXCRETION IN THE DOG

EWALD E. SELKURT, L. JAMES TALBOT¹ AND C. RILEY HOUCK

From the Department of Physiology, New York University, College of Medicine

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It has been demonstrated by Sutton, Kaeser and Hansard (1) that the synthetic estrogenic substance, diethylstilbestrol, causes enhanced urinary excretion of ascorbic acid by rats, animals capable of synthesizing ascorbic acid. The possibility that this might be due to alteration of normal renal tubular function is suggested by the finding of Thorn and Engel (2) that estradiol influences the excretion of sodium and potassium chlorides in dogs. According to these authors, the immediate effect of parenteral administration of estradiol is a decreased excretion of sodium chloride and an increased excretion of potassium chloride, a fact which suggests decreased tubular reabsorption of the latter.

The purpose of the present investigation was to determine if the estrogen, estradiol benzoate, could increase the urinary excretion of ascorbic acid in the dog, which also synthesizes vitamin C. Further, the possibility that such increased urinary excretion might be due to an altered renal mechanism was examined. This could result from a decreased tubular reabsorption of ascorbic acid, a threshold substance cleared by the kidney by glomerular filtration and tubular reabsorption, the latter being limited by a maximal rate (T_m) (3).

It is our experience that the maximal rate of tubular reabsorption (T_m) of ascorbic acid in the dog not only lends itself to fairly precise measurement, but is capable of duplication over a considerable period of time when load levels are adequate to effect tubular saturation (see table 1). In this we are in agreement with Ralli and her associates (3). Accordingly, it is possible to combine daily observations of urinary excretion of ascorbic acid with measurements of ascorbic acid T_m , in order to determine whether increased or decreased excretion is related to changes in tubular function.

METHOD. Four female dogs, ranging in weight from 13.5 to 19.0 kgm., were used. One of the dogs was castrated. Repeated clearances of three to six urine collection periods of ten minutes each were made at various levels of plasma ascorbic acid concentration before, during, and, in two dogs, after injection of estradiol. In all cases the control clearances were made within fifty days before the injection of the hormone. Usually, the desired plasma levels were obtained by constant intravenous infusion, but in some cases subcutaneous injections were used. The simultaneous clearance of creatinine was taken as an index of glomerular filtration rate. Tubular reabsorption of ascorbic acid (mgm./min.) was calculated as the difference between ascorbic acid load (glomerular filtration rate \times plasma concentration of ascorbic acid) and its concurrent rate of excretion (urinary concentration \times urine flow in cc./min., or UV).

¹ Fellow in Medical Sciences, National Research Council.

These calculations assume that there is no plasma binding of ascorbic acid, which assumption is supported by the fact that at high plasma levels the ascorbic acid

TABLE 1

Ascorbic acid Tm in the dog before, during, and after treatment with estradiol benzoate in a representative experiment (dog RG, wt., 18 kgm.)

DATE	PERIODS	PLASMA ASCORBIC ACID	FILTRATION RATE	LOAD	UV	Tm	$\frac{CAA^*}{CCr}$
Before							
12-19-42	3	mgm. per cent 2.45	cc./min. 71.0	mgm./min. 1.733	mgm./min. 1.158	mgm. reabs. per min. 0.575	0.670
12-29-42	4	4.01	67.0	2.692	2.094	0.598	0.776
	4	5.37	78.0	4.200	3.594	0.646	0.825
1-6-43	4	2.85	68.0	1.938	1.528	0.410	0.786
1-8	4	3.93	76.0	3.000	2.500	0.500	0.856
1-9	4	2.50	94.0	2.350	1.838	0.512	0.790
1-12	4	2.37	77.0	1.815	1.330	0.485	0.750
1-14	3	3.40	70.0	2.366	1.738	0.628	0.736
1-16	3	1.58	91.0	1.423	0.913	0.510	0.638
1-21	4	3.95	81.0	3.190	2.535	0.655	0.800
Average			77.3	2.471	1.919	0.552	0.763
During							
1-23	4	2.34	100.0	2.340	2.076	0.264	0.880
1-26	3	2.78	103.4	2.880	2.530	0.350	0.880
1-30	3	2.51	74.0	1.860	1.797	0.063	0.974
1-30	4	2.56	85.0	2.155	2.036	0.119	0.944
2-2	4	4.01	86.6	3.465	1.584	0.575†	0.832
2-3	3	2.32	80.0	1.850	2.890	0.266	0.857
2-6	4	1.50	110.4	1.657	1.462	0.195	0.881
Average			91.3	2.315	2.054	0.261	0.892
After							
2-10	4	2.03	75.0	1.520	1.220	0.300	0.808
2-13	4	1.93	81.0	1.572	1.201	0.370	0.764
2-17	4	2.72	87.0	2.370	1.910	0.460	0.808
2-20	4	3.33	65.4	2.171	1.808	0.362	0.830
Average			77.1	1.908	1.535	0.373	0.802

* $\frac{CAA}{CCr} = \frac{\text{Ascorbic acid}}{\text{Creatinine}}$ clearance ratio.

† During estradiol treatment excessively high load levels gave values for Tm approximating the normal. See discussion.

clearance approaches the filtration rate (3). It was found that a load/Tm ratio of over 2.5 was required to effect complete tubular saturation. Urines were

collected by catheter, and the bladder was washed after low urine flows. The dogs received 30 to 40 cc./kgm. of water prior to the clearance study to insure adequate urine flow.

Plasmas were precipitated for creatinine determination by the CdSO_4 method of Fujita and Iwatake (4), and the creatinine concentration of the plasma filtrate and diluted urines was determined by the method of Folin and Wu (5). Ascorbic acid was determined in both plasma and urine by the dichloroindophenol method of Mindlin and Butler (6)² using a photoelectric colorimeter, with modifications to correct for turbidity as suggested by Bessey (7). In dog F, simultaneous clearances of mannitol and creatinine were made to check on the presumption that creatinine accurately measured the filtration rate during estradiol action. Mannitol determinations were done by the method of Smith, Finkelstein and Smith (8). All analyses were made in duplicate.

Three dogs (two normal and one castrate) were kept in metabolism cages for the determination of the hourly excretion of ascorbic acid before, during, and after treatment with estradiol. To minimize deterioration during 24-hour collections, 5 cc. of concentrated H_2SO_4 were added to the collection vessels, giving a final urine pH of 1.25 to 4.0, depending on the urine volume. Although appreciable deterioration of ascorbic acid occurs when urines are kept under these conditions for long periods at room temperature, nevertheless comparison can be made between control and experimental collections obtained under the same conditions. When ascorbic acid clearances were made during the total urine collection period, the latter was interrupted for an interval of 18 to 30 hours to allow the plasma level of ascorbic acid to return to normal before collections were resumed. Fasting plasma determinations of ascorbic acid were regularly made about 16 hours after feeding. The diet was kept constant in amount during total urine collection periods.³

Since alkalinity influences the rate of destruction of ascorbic acid in urine (9), it was necessary to determine whether estradiol treatment altered urinary pH significantly. A Coleman pH meter was used for this purpose. Regular hematocrit determinations were made on all dogs.

After suitable control periods, estradiol benzoate (Progyon-B)⁴ in sesame oil was injected intramuscularly in the daily dosages listed in table 2. Dog RG received a total of 23.4 mgm. in 14 days; dog S, 30.0 mgm. in 13 days; dog P, 21.7 mgm. in 13 days. Dog F, in which hourly excretion studies were not made, and which does not appear in the table, received 0.123 mgm./kgm. for 11 days (total, 18.3 mgm.).

² Three cubic centimeter aliquots of plasma and urine were added to 17 cc. of 3.0 per cent HPO_3 as soon as obtained. Protein precipitation was followed by centrifuging and filtering, and urines were further diluted with acid before colorimetric analysis. The high dilution needed for the urines eliminated the action of other reducing substances and allowed them to be analyzed in the same manner as the plasma filtrates.

³ Daily rations of 200 to 250 grams were given of a mixture of the following proportions: 100 grams cracker meal; 30 grams skim milk; 10 grams brewer's yeast; 4 grams $\text{Ca}_3(\text{PO}_4)_2$; 3 grams of salt mixture (10 parts NaCl, 1 part Fe-citrate, and 4 parts Mg-citrate); and 5 cc. of cod liver oil.

⁴ Courtesy of Dr. Max Gilbert, Schering Corp.

RESULTS. A. *Urinary ascorbic acid excretion.* Changes in hourly excretion are summarized in table 2. For economy of space, the collection intervals have been divided into convenient periods of 4 or 5 days in length, and the values given represent the average of all determinations made during these periods.

B. *Tubular reabsorption.* Since the tubular reabsorption of ascorbic acid increases with the glomerular load up to saturation of the tubules, changes in

TABLE 2

Effect of estradiol benzoate on ascorbic acid excretion, water balance, fasting plasma ascorbic acid, and hematocrit in normal and castrate dogs

(All values are averages of determinations done during 4 or 5 day periods)

NUMBER OF DAYS IN PERIOD	ESTRADIOL INJECTED DAILY	ASCORBIC ACID EXCRETION	PLASMA ASCORBIC ACID	URINE FLOW	WATER INTAKE	HEMATOCRIT
Dog S (19 kgm.)						
	mgm./kgm.	mgm./hr.	mgm. per cent	cc./hr.	cc./hr.	per cent
5	Control	3.41	0.48	52.5	75.0	35.4
4	0.088	4.20	0.50	56.0	108.0	35.5
4	0.088	4.95	0.32	70.0	107.0	32.5
5	0.171	5.26	0.30	75.0	119.0	30.5
5		5.91		74.3	100.0	26.5
5		5.82	0.48	57.0	100.0	11.0
5		4.70	0.35	52.8	77.0	16.0
Dog RG (18 kgm.)						
2	Control	4.87	0.45	37.0		35.0
4	0.0925	5.18	0.31	31.2		42.0
5	0.0925	8.70	0.42	67.4		37.5
5	0.0925	7.72	0.32	79.0		36.2
5		6.61	0.30	41.0		31.2
5		4.34	0.35	52.2		28.0
5		4.93	0.33	36.0		30.5
Dog P (castrate) (14 kgm.)						
4	Control	4.77	0.39	27.7	43.0	35.0
5	0.075	3.55	0.37	24.8	39.0	43.0
4	0.087	4.37	0.36	24.4	41.2	44.0
4	0.111	4.59	0.30	22.4	38.0	39.0
4	0.111	5.67	0.32	23.0	38.0	35.5
4		6.11	0.40	27.5	39.0	26.7

reabsorptive capacity resulting from estradiol treatment must be compared with normal values in terms of the load at which reabsorptive activity is measured. Accordingly, control and experimental data are presented in figure 1 in terms of T/T_m ratios as plotted against load/ T_m ratios, where T is the observed reabsorptive activity at a particular load and T_m is the maximal reabsorptive capacity, as independently measured at load values high enough to assure saturation. The control data on four dogs, expressed as T/T_m in relation to load/ T_m , have been combined to give a mean curve which is taken as showing

the normal relationship between load and reabsorptive activity. This relationship can be spoken of as the ascorbic acid "titration curve," in the sense in which Smith and his co-workers (10) have spoken of the glucose and diodrast "titration curves" in the human kidney.

It is apparent that considerable splay is present in this titration curve, as compared with the curve to be expected in the case of a substance completely reabsorbed by the tubules at all load levels up to tubular saturation. It is also apparent that a load/ T_m ratio of over 2.5 is required to obtain complete saturation.

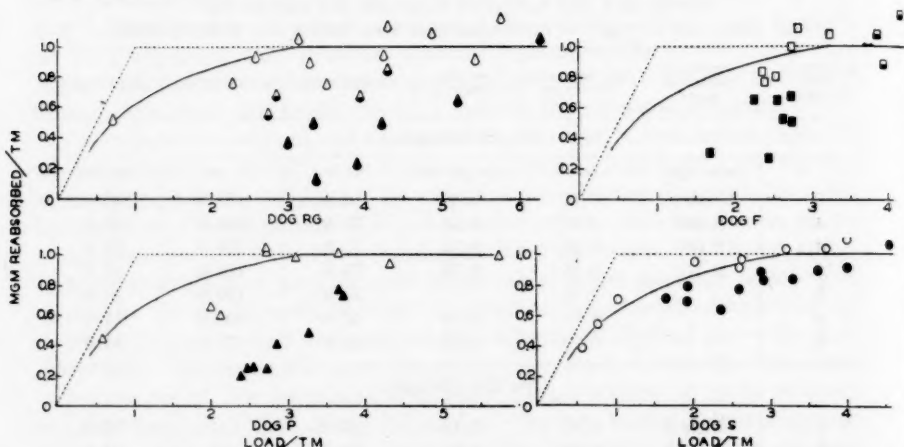


Fig. 1. Effect of estradiol benzoate on ascorbic acid reabsorptive capacity, as expressed in terms of T/T_m plotted against load/ T_m , where T is the observed reabsorptive capacity at any particular load, and T_m is the maximal reabsorptive capacity independently measured at load values high enough to assure saturation. Open symbols, control; solid symbols, during estradiol treatment; semi-solid symbols, recovery. Each symbol represents a clearance experiment into which are averaged three to six consecutive urine collection periods.

Control data have been combined in a mean "titration" curve which is taken to show the normal relationship between load and reabsorptive capacity. This curve was obtained by approximation of T/T_m ratios averaged in blocks of load/ T_m ratios. The dotted line shows the relationship to be expected for a substance completely reabsorbed by the tubules up to tubular saturation.

Because the effects of treatment differed in the various dogs, each is shown separately in figure 1, in reference to the above mean normal curve. T_m values during estradiol treatment are summarized in table 3, and in figure 1 it will be seen that in the treated animals reabsorption is reduced at loads which normally effect tubular saturation.

The decrease in tubular reabsorption increases the plasma clearance of ascorbic acid so that in some cases it approaches the creatinine clearance (fig. 2). The increased clearance tends to reduce the fasting plasma level of ascorbic acid during treatment (table 2).

C. *Glomerular filtration rate.* Increases of 13, 18, and 23 per cent in the average glomerular filtration rate were noted in dogs F, RG, and S, respectively. These changes may have resulted from expansion of the plasma volume, as indicated by a greater water intake proportional to urinary output early in the treatment (see dog S, table 2).

D. *Urine pH.* The control pH of the urine of dog F averaged 5.79 (25 observations), and in the experimental periods, 5.96 (28 observations). The absence of change in the urine pH during treatment indicates that this factor is not important in influencing the excretion of ascorbic acid in this study.

TABLE 3

The effect of the administration of estrogen on the ascorbic acid reabsorptive mechanism of the kidney in the dog

	Tm (MG./MIN.)			
	Dog F	Dog RG	Dog S	Dog P (castrate)
Control				
Range.....	0.485-0.671 (4)*	0.410-0.655 (9)	0.538-0.645 (6)	0.533-0.595 (6)
Average.....	0.600	0.542	0.590	0.571
During treatment				
Range.....	0.159-0.405 (5)	0.063-0.575 (7)	0.373-0.623 (10)	0.110-0.436 (8)
Average.....	0.314	0.262	0.488	0.235
After treatment				
Range.....	0.529-0.728 (3)	0.300-0.460 (4)	†	‡
Average.....	0.635	0.373		
Per cent decrease during treatment.....	48.0	52.0	17.0	59.0

* Numbers in parentheses denote the number of clearance observations entering into the average Tm. Each clearance observation consisted of from three to six continuous urine collection periods.

† Recovery not followed.

‡ Dog sacrificed before recovery.

E. *Creatinine and mannitol clearance.* While estradiol was being given, the creatinine/mannitol clearance ratio averaged 1.03 for 16 urine collection periods. Thirteen periods ranged from 0.97 to 1.03, but three (all during one series of clearance determinations) were unaccountably high, i.e., as high as 1.17.

F. *Blood and vascular changes.* Other workers (11) have reported in detail certain changes which we have noted. All dogs showed increased capillary fragility and petechial hemorrhages, and the clotting time was noticeably increased. Severe anemia resulted in the death of two of our dogs, F and S, several weeks after the injections were stopped.

G. Kidney histology. Kidneys were obtained from dog S twenty days after the last injection of estradiol, when she died from anemia. Except for post-mortem changes, sections of these kidneys appeared normal. A kidney was removed from dog P under anesthesia while receiving a daily injection of 0.111 mgm./kgm. of estradiol and during a maximal effect on ascorbic acid excretion and tubular reabsorption. Sections of this kidney also appeared normal.⁵

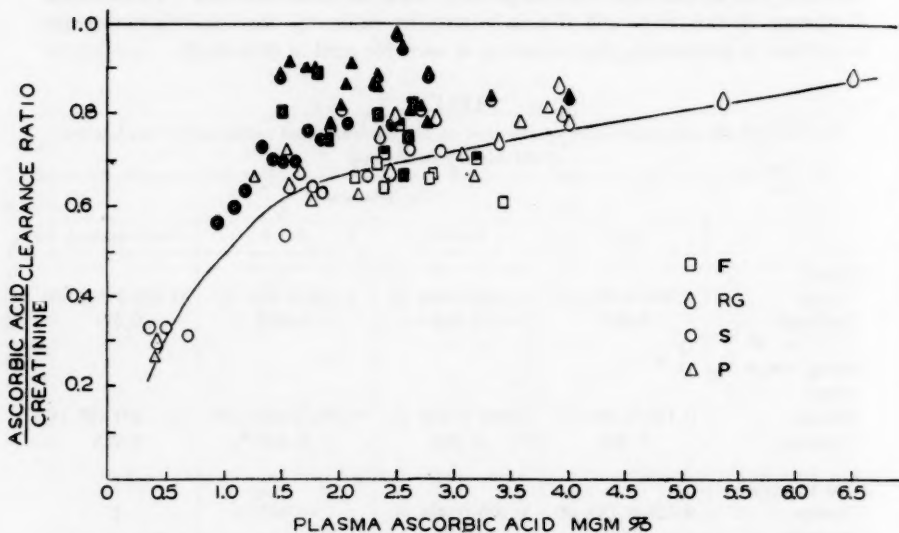


Fig. 2. Effect of estradiol benzoate on the renal plasma clearance of ascorbic acid at various plasma levels, as expressed in terms of ascorbic acid/creatinine clearance ratios. Open symbols, control; solid symbols, during estradiol treatment; semi-solid symbols, recovery. Each symbol represents a clearance experiment into which are averaged three to six consecutive urine collection periods.

The mean normal curve was obtained by approximation of clearance ratios averaged in blocks of plasma ascorbic acid concentration.

DISCUSSION. The increased urinary excretion of ascorbic acid following injections of estradiol benzoate in the dog appears to be due to a decreased renal tubular reabsorption of this substance. The decreased tubular reabsorption increases the renal plasma clearance so that in some cases the latter approaches the creatinine clearance (fig. 2). Hence depletion of plasma ascorbic acid occurs, and this presumably leads to acceleration of ascorbic acid synthesis, thereby further increasing the rate of excretion through maintenance of the plasma level.

⁵ The only significant alteration noted in the kidney of dog P concerned the renal arterioles, which were universally thick and with small lumina. The cells of the media appeared large, fairly clear, and sometimes in two layers. The so-called juxta-glomerular apparatus was prominent whenever encountered; no granules were seen in its cells. In some areas there was a slight increase in interstitial connective tissue surrounding a few tubules and more rarely the glomerulus.

It is interesting to note that considerable ascorbic acid is excreted by the dog at low plasma levels, indicating that tubular reabsorption is never complete, a phenomenon also evident in man (12). Incomplete tubular reabsorption with increasing load levels is reflected in the splay in the ascorbic acid titration curve (fig. 1). Smith and associates (10) report a slight splay in the glucose titration curve of the human kidney, and on the explicit premise that glucose reabsorption is complete in each nephron up to the load at which saturation occurs, accept this splay as reflecting variations in the ratio of *filtration rate/glucose reabsorptive capacity* in individual nephrons. The splay in the ascorbic acid titration curve may thus reflect a disparity in the ratio: *filtration rate/ascorbic acid reabsorptive capacity* in individual nephrons. On the other hand, such a splay may reflect an intrinsic characteristic of the reabsorptive activity of the tubule cells with respect to ascorbic acid, whereby the efficiency with which ascorbic acid is removed from the tubular urine decreases as its concentration in the urine is increased. A choice between these alternatives cannot be made from the present evidence.

The fact that the splay appears to be so markedly accentuated by estradiol treatment, without any large change in the total filtration rate, suggests that estradiol affects the kinetics of the tubular reabsorptive process rather than by significantly altering the ratio of *filtration rate/ascorbic acid reabsorptive capacity*. This inference is supported by the fact that T_m is unchanged by treatment, although a greater load/ T_m ratio is required to effect saturation. It is further supported by the fact that the changes in the titration curve are not accompanied by histological changes in the renal tubules, and by the rapid reversibility of the changes in reabsorptive activity.

The means by which estradiol alters the tubular mechanism cannot at present be answered. One of the most striking changes seen in treated dogs is a marked depression of the hematocrit. However, the renal effect in all of our dogs occurred before the hematocrit had been greatly decreased, and tended to return to normal after treatment, when the hematocrit was invariably low. It therefore does not appear that anemia itself affects the renal mechanism.

Petechial hemorrhages and tendency to bleed after slight trauma were noted in all of the treated dogs. This apparent increase in capillary fragility may well be the result of enhanced ascorbic acid loss from the body, brought about by the altered renal mechanism which increases the plasma clearance of this substance.

SUMMARY AND CONCLUSIONS

1. In the normal dog, tubular reabsorption of ascorbic acid is incomplete at plasma levels considerably below those required to effect complete saturation of the tubules. Consequently, ascorbic acid is excreted in the urine at all plasma levels.

2. By reducing tubular reabsorption, estradiol benzoate increases the clearance of ascorbic acid. As a result, the rate of excretion is increased while the plasma level tends to be reduced.

3. During estradiol benzoate treatment, load levels considerably higher than those normally needed for tubular saturation produced a maximal rate of reabsorption equivalent to the normal. In the absence of any large change in glomerular filtration rate and with no significant histological changes, this was taken to mean that estradiol affects the kinetics of ascorbic acid reabsorption, rather than by changing the ratio of *filtration rate/reabsorptive capacity*.

4. Changes in tubular reabsorption are not attributable to changes in urine pH, decreases in hematocrit, or visible tubular damage.

5. Changes in water balance and slight increases in glomerular filtration rate were observed in normal dogs after estrogenic treatment, but not in one castrate. However, all showed a comparable alteration of the renal ascorbic acid mechanism.

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NEUROMUSCULAR TRANSMISSION IN THE SINGLE NERVE AND MUSCLE FIBER PREPARATION

S. E. STEIMAN¹

From the Department of Physiology in the Harvard Medical School

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Within the past few years evidence has accumulated which supports the view that acetylcholine is the chemical transmitter of nervous impulses at the neuromuscular junction. The theory of chemical transmission has been based thus far on the results obtained from the whole muscle with its nerve supply. As yet, no systematic study has been made on the intact, innervated, single muscle fiber. It appeared desirable, therefore, to attempt this study.

METHODS. Frogs (*R. pipiens*) were used. The sartorius with its nerve supply was isolated and kept in modified Ringer (NaCl, 0.67 gram; KCl, 0.02 gram; CaCl₂, 0.02; gum acacia, 0.01; NaHCO₃, 0.02 gram; glucose, 0.02 gram; H₂O, 100 cc.). The nerve-sartorius preparation was then placed in a shallow pool of Ringer on a glass plate and the muscle, with its dorsal surface uppermost, fixed at both ends. The preparation was transilluminated and with the aid of a dissecting microscope a single muscle fiber was isolated. The technique used was a modification of the method described by Kato (1934). First, layers of muscle fibers were carefully peeled off with a fine iridectomy knife until only two or three layers remained. The muscle was then turned over with its ventral surface uppermost. In this aspect, the courses of the nerve branches could be seen readily. The nerve was stimulated with induction shocks to determine a group of active muscle fibers. All nerve twigs were then cut except the one going to these fibers. The latter were then teased apart at the upper pelvic end of the muscle. Test shocks were frequently applied to the nerve so that the active fibers could be identified. After one had been chosen, the others were cut. These fibers were next cut away on either side of the entrance of the nerve twig to the active fiber. The small remnants of muscle tissue (about 3 to 5 mm.), however, did not respond to either direct or indirect stimulation. No attempt was made to isolate the entire length of the fiber. At the pelvic and tibial ends a small number of cut muscle fibers were left and these helped to fix the active intact fiber. The remnants at either end also did not respond to stimulation since all nerve branches innervating them were cut.

The preparation was next placed in a Petrie dish filled with paraffin in which two small troughs at right angles had been made. The muscle fiber was placed in one trough and in the other the nerve lay across two small silver electrodes. The muscle fiber was put under a small amount of tension and both ends fixed. The responses of the fiber were recorded by the mercury droplet method of Pratt and Eisenberger (1919). The fiber was sprayed with fine mercury globules and the preparation then placed under a microscope. A globule was strongly

¹ National Research Council Fellow in the Medical Sciences.

illuminated and a demonstration ocular projected the reflected ray of light from the droplet onto a moving film. The muscle fiber could be visualized directly through the regular eye-piece of the microscope.

In some experiments a single muscle fiber from the retrolingual membrane of the frog was used. The preparation was made according to the method of Pratt and Reid (1930). A nerve filament was found and traced to a muscle fiber. The former was cut proximally and all other nerve filaments that were found were severed. In addition, all other muscle fibers in the vicinity of the one that was being recorded were also cut. "Unipolar" stimulation was used to activate the nerve. One electrode was a quartz-covered platinum wire (about 5μ diameter) placed on a micromanipulator, and the other was a large indifferent electrode placed in the bath covering the preparation. That the nerve was stimulated and not the muscle fiber was determined by placing the electrode close to the muscle fiber and stimulating it with the same strength of stimulus effective for the nerve. No response was obtained as the threshold for the muscle fiber was higher than that of the nerve filament.

The nerve was stimulated supra-maximally at various frequencies with condenser discharges. The drugs employed were acetylcholine chloride (Merck), eserine salicylate (Sharpe and Dohme), and curare (the crude drug).

RESULTS. I. Fatigue. It is well known that in stimulating a nerve-muscle preparation indirectly with tetanizing currents fatigue occurs first at the neuromyal junction, since the nerve can still conduct impulses and direct stimulation of the muscle elicits a contraction. Asmussen (1934) stimulated small bundles of muscle fibers indirectly and found that, with the onset of fatigue, the tension curve fell in a stepwise manner. That this effect was not due to fatigue of the muscle fibers themselves was shown by the response obtained to direct stimulation. Moreover, direct stimulation of a similar small bundle of muscle fibers resulted in a tension curve which fell smoothly with the onset of fatigue. On the basis of these results Asmussen concluded that the step-like fall of tension during indirect stimulation was due to an all-or-none behavior of the end-plate. Accordingly, it appeared desirable to test this conclusion directly with a single motor end-plate.

Figure 1 illustrates the results obtained in a typical experiment. A single muscle fiber was continuously stimulated indirectly at the rate of 5 per second. There was a progressive decline in the height of contraction (cf. A and B). However, the fiber responded to each stimulus for 1 minute 16 seconds after the start of stimulation. The fiber then suddenly failed to respond to a stimulus but did contract to the next eight stimuli when it again suddenly failed to contract. It will be observed from the subsequent sections of the record (C to F) that, at first, the failure of the muscle fiber to respond occurred infrequently. The interval between responses became progressively longer and after 5 minutes of stimulation a period of 12 seconds or more was noted between two successive responses.

When the fiber first began to fail to respond there was little change in the height of contraction, but as this failure became more frequent and more pro-

longed there was a progressive increase in the height of response until finally it approached that obtained at the start of stimulation (fig. 1A and F). A stimulus well above threshold was used throughout these experiments and at no time during the period of stimulation did a further increase in strength of stimulus have any effect on the responses of the muscle fiber.

Indirect stimulation of a single muscle fiber with frequencies between 100 and 155 per second produced, initially, a tetanus for 1 to 2 seconds followed by a fall in tension. There then appeared single responses at irregular intervals which became longer as stimulation continued. However, with these higher frequencies the responses soon ceased and did not return even though stimulation was continued from 1 to 5 minutes longer. Increase of strength of stimulus was also without effect. That the muscle fiber itself was not fatigued was shown by eliciting a contraction to a direct stimulus at varying times after the responses to indirect stimulation had stopped.

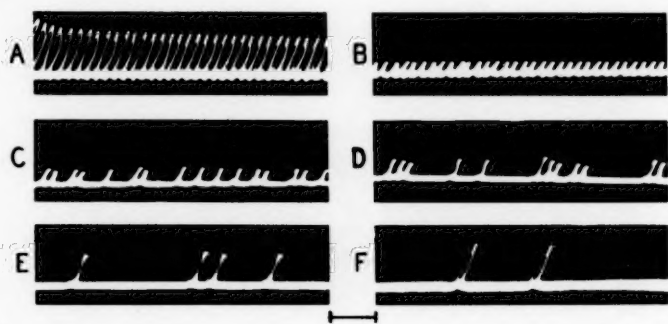


Fig. 1. Fatigue. Mechanical responses of a single muscle fiber stimulated continuously via its nerve at the rate of 5 per sec. A: beginning of stimulation; B to F: 1, 2, 2.5, 3.5, and 4.25 minutes later, respectively.

Direct stimulation of a single muscle with frequencies between 5 and 10 per second did not result in any sudden failure to respond as observed with indirect stimulation. The fiber responded to each stimulus and with the onset of fatigue there was a continuous decrement in the height of contraction (see Asmussen, 1934). This smooth decline in tension continued until the fiber was completely fatigued at which time there was no further response. With continued stimulation there was no return of the contraction. Here again increase of the stimulus, which was initially well above the threshold for the fiber, was also without effect. In some cases a nerve-muscle fiber preparation was used and the muscle fiber directly stimulated until it was completely fatigued and did not contract. At this point the muscle fiber was stimulated through its nerve but this also failed to produce a response. That the nerve fiber and neuromyal junction were intact in these experiments was shown by allowing the muscle fiber to recover, after which indirect stimulation resulted in contraction.

The results obtained from stimulating indirectly 2 to 4 muscle fibers confirm those obtained by Asmussen (1934). In a typical experiment 2 muscle fibers

were stimulated indirectly at the rate of 4 per second. The general curve followed that of figure 1. However, both fibers did not fail to respond at the same time. Instead, one fiber dropped out suddenly and the height of response was about half of the previous contraction involving the two fibers. The fiber would then respond intermittently. Soon the other fiber began to fail to contract and at this time there appeared intervals during which there was no response to some of the stimuli. When a contraction did occur it consisted of either one or both fibers.

This step-like effect was even more strikingly brought out by stimulating 4 muscle fibers indirectly. At first all the fibers contracted but with further stimulation each fiber began to drop out suddenly and then also responded intermittently. Four step-like gradations could be observed on the record. At times a stimulus failed to produce a response of any of the 4 fibers while the next effective stimulus might result in a response of any or all of the muscle fibers. The intervals between these contractions were not as long as those seen with the single muscle fiber as at least one of the fibers would soon respond.

II. *Eserine*. Most workers have been unable to observe any potentiation of the muscular response to single nerve volleys by eserine in amphibia. Thus

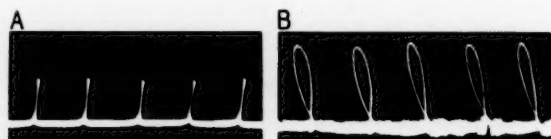


Fig. 2. *Eserine*. Single muscle fiber stimulated indirectly every 10 sec. A: before eserine. B: 5 min. after the addition of 0.3 mgm. eserine to the Ringer solution bathing the preparation.

Kruta (1935) employing eserine, and Cowan (1936) using prostigmine, reported negative results. Brown (1937b) also found that eserine (1 to 100 γ) perfused through the vessels of the isolated frog's leg or delivered in the circulating blood produced no augmentation of the response. However, Feng (1937) was able to obtain potentiation to single shocks in the isolated nerve-sartorius preparation and Hodes and Steiman (1939) confirmed this potentiation effect by injecting eserine into the dorsal lymph sac.

In the present experiments it was also found that eserine would potentiate the response of a single muscle fiber indirectly stimulated once every 10 seconds (fig. 2). Doses of eserine which ranged from 0.1 to 0.3 mgm. were added to the Ringer solution bathing the preparation. In all the preparations tested (8) there was approximately a 100 per cent increase in the height of contraction (cf. A and B, fig. 2).

With faster frequencies of stimulation (2 to 10 per sec.) after eserine, the fiber responded to the first few stimuli and then suddenly ceased to contract although stimulation was continued from one to two minutes or more. Frequently, the fiber again contracted after it had failed to respond to one or two stimuli. Occasionally there appeared, 10 to 15 seconds after the last response, another contraction and none after that.

III. *Acetylcholine and curare.* Dale, Feldberg and Vogt (1936) showed that acetylcholine is released at neuromuscular junctions on stimulation of the motor nerve. Moreover, in normal mammalian muscle, close arterial injections of acetylcholine produce a response that can be identified as a true propagated contraction by the accompanying outburst of action potentials (Brown, Dale and Feldberg, 1936; Brown, 1937a). However, an excess of acetylcholine will paralyze muscles instead of exciting them (Dale, Feldberg and Vogt, 1936; Rosenblueth, Lindsley and Morison, 1936). This fact, together with other experimental results, led Rosenblueth and Morison (1937) to postulate a paralytic level of acetylcholine. On the basis of the all-or-none law it should be expected, therefore, that as soon as the paralytic threshold is reached the single muscle fiber should suddenly cease to contract. That this is actually the case can be seen in figure 3. A single muscle fiber was indirectly stimulated every 2 seconds and gave the characteristic all-or-none response. After addition of 30 γ acetylcholine to the Ringer solution the fiber continued to contract until the diffusion



Fig. 3. Acetylcholine. Single fiber stimulated indirectly every 2 sec. at arrow 30 γ of acetylcholine were added to the bathing solution.



Fig. 4. As in figure 3, but records from 4 muscle fibers. At each arrow 15 γ of acetylcholine were added.

of acetylcholine reached a paralytic concentration at the end-plate and the fiber failed to respond to the next stimulus. A subsequent stimulus again elicited a contraction and there was no further response for 20 seconds. At the end of this time the contractions reappeared and these were of the same magnitude as those shown in the record.

Figure 4 illustrates the behavior of 4 muscle fibers to excess acetylcholine. A paralytic concentration was not reached at the 4 end-plates simultaneously and so there occurred a step-like gradation of the response as the individual fibers dropped out. In the record there are 4 distinct gradations of the total response, indicating further that each muscle fiber dropped out suddenly.

The all-or-none behavior of the motor end-plate is again demonstrated by the action of curare (fig. 5). After the addition of curare the responses continued until a concentration sufficient to block transmission was reached when the muscle fiber suddenly failed to respond. Although stimulation was continued for 2 min. or more there was no further contraction. Direct stimulation, however, was effective.

Acetylcholine has been shown to have a decurarizing effect on mammalian muscle (Briscoe, 1936; Rosenblueth, Lindsley and Morison, 1936). Hodes and Steiman (1939) were unable to demonstrate the decurarization effect of acetylcholine in the frog except in a few cases. In the present study the writer also was unable, in most instances, to obtain a decurarization by acetylcholine. However, in a few experiments, doses of 15 to 50 γ acetylcholine added after the fiber was curarized resulted in one or two contractions after which there were no more responses. Attempts to repeat this result by adding more acetylcholine to the Ringer's were unsuccessful. It appeared that the optimal condition for obtaining the decurarization effect was a degree of curarization where a stimulus just failed to produce a response. The exact dose of acetylcholine was difficult to determine for a given preparation and the stage of curarization where a contraction would result could be easily missed.

DISCUSSION. The results obtained on single-fiber nerve-muscle preparations (figs. 1 to 5) confirm the results and inferences of previous studies on large groups of fibers. The process of neuromuscular transmission follows the all-or-none law because the first step of muscular activity—the propagated disturbance—is itself quantal. When repetitive stimulation (fig. 1) or excess acetylcholine



Fig. 5. Curare. Single muscle fiber stimulated indirectly every 2 sec. Curare was added at the beginning of the record (arrow).

(figs. 3 and 4) or curare (fig. 5) leads to a failure of transmission this failure is sudden in the single fiber.

The data on fatigue (fig. 1) support the distinction made in previous studies from this laboratory (Rosenblueth and Luco, 1939; del Pozo, 1942) between transmission and contraction fatigue. Thus, the decrease of response from A to B in figure 1 corresponds to contraction fatigue, since transmission has not failed. The increase of contractions from D to F, on the other hand, corresponds to a recovery from fatigue of the contractile systems, a recovery which occurs because the progressive development of transmission fatigue leads to relatively long rest intervals between the responses.

The random appearance of twitches in figure 1 C to F, after transmission fatigue first became manifest, suggests random changes in one or more of the factors involved in the process of transmission. These random variations have also been observed in whole muscle experiments both during the 4th stage (fatigue) and during the later 5th stage of relative recovery of transmission (Rosenblueth and Luco, 1939).

All the phenomena studied have been satisfactorily explained by the theory of neuromuscular transmission mediated by release of acetylcholine at the motor nerve endings.

Transmission fatigue (fig. 1) is due to a decrease of the quanta of acetylcholine per nerve impulse. Potentiation by eserine (fig. 2) is due to persistence of acetylcholine at the junction, protected by the eserine from the destruction promoted by cholinesterase. Block of transmission by acetylcholine (figs. 3 and 4) results from excess of the mediator. Block by curare (fig. 5) is due to the fact that the drug raises the threshold of muscle to acetylcholine. The experiments are not crucial, however, with respect to theories of transmission—i.e., other interpretations would cover the data.

SUMMARY

In a single muscle fiber indirectly stimulated at various frequencies transmission fatigue and contraction fatigue appear as independent phenomena (fig. 1).

The neuromuscular junction reacts in an all-or-none manner to fatigue (fig. 1), eserine (fig. 2), acetylcholine (figs. 3 and 4) and curare (fig. 5).

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THE KIDNEY AS A SOURCE OF GLUCOSE IN THE EVISCERATED RAT^{1, 2}

ROGER M. REINECKE

From the Division of Physiological Chemistry, University of Minnesota, Minneapolis

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Bergman and Drury (1938) demonstrated that the presence of functional kidneys led to a decrease in the apparent rate of utilization of glucose by the eviscerated rabbit. The author (Reinecke, 1942) found that if no sugar were given, the non-nephrectomized, eviscerated rat would survive much longer than the nephrectomized, eviscerated rat. Since these animals probably die of hypoglycemia, this finding, too, suggested that the kidney was of importance in carbohydrate metabolism. In an effort to elucidate just what the rôle of the kidney may be, the experiments reported here were undertaken.

METHODS AND MATERIALS. Male albino rats obtained from Sprague-Dawley, Inc. were used. They were eviscerated when about 300 grams in weight, and were allowed to go without food for about 24 hours before being subjected to the operation. Prior to this they were maintained on tap-water and Purina "Whelping Fox Chow" or Alber's "Friskies" given ad libitum.

Evisceration. Since the operative procedures are of critical importance in these experiments, they are described in some detail. In order to obtain the best possible collateral circulation for the segment of vena cava that traverses the liver, this vessel is tied off just caudal to the liver in a preliminary operation performed when the rat is weaned or shortly thereafter. With the animal under ether anesthesia, the peritoneal cavity is opened by a ventral midline incision starting at the tip of the ensiform process and extending about 1.5 cm. caudally. The intestines are pushed to the animals' left and caudally by a flat instrument such as a scalpel handle. A thumb forceps with rather wide tips is then used as a double bladed retractor to maintain the exposure of the caudal portion of the vena cava where it enters the liver. A ligature of size "A" serum proof silk is passed through the thin mesentery suspending the liver from the dorsal surface of the peritoneal cavity at a point just slightly cranial to the entrance of the vena cava into the liver substance. This ligature is then used to tie off the vena cava at the level where it enters the liver. The incision in the abdominal wall is closed in two layers with size "A" silk. Usually the operative mortality is less than 5 per cent. If the ligature is placed in a more caudal position, the right kidney will often be found to be completely atrophic at the time of evisceration. Placing the ligature more caudally, furthermore,

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tends to cause more bleeding with the consequent formation of troublesome adhesions. The formation of adhesions is also favored by the use of heavier silk or by excessive handling of the intestines such as would occur if they were temporarily removed from the abdominal cavity. Sterile procedure is unnecessary, but sulfonamides may be used with advantage in closing the abdominal wall. They should not, however, be placed in the peritoneal cavity for they tend to cause adhesions.

The evisceration proper is performed under ether anesthesia. The abdomen is opened along the midline ventrally from the base of the ensiform process to the level of the prevesical space. The intestine is retracted to the animal's right and the coeliac and superior mesenteric arteries are exposed to their origins from the aorta by blunt dissection and are tied with a single ligature. (In about 5 per cent of the animals these arteries are found to arise as a common trunk.) The esophagus is then connected to the rectum by a 3-5 cm. length of no. 8 French rubber catheter that has been fitted with a glass cannula at either end. This procedure improves the viability of the preparation by preventing the overflow of mucus into the pharynx and its consequent interference with respiration. The cannulae are tied into place in such a manner as to prevent bleeding from the esophageal and colonic stumps. The caudal cannula is introduced into the descending colon through a rent made opposite the mesenteric attachment. It is advanced caudally until it is at the level at which the inferior mesenteric artery turns cranially to run alongside the colon. It is then tied in place with a ligature that also includes all of the vessels that lie parallel to the colon. The cranial cannula is introduced into the esophagus via a rent made in the stomach near the cardia. It is tied in place with a ligature that serves in addition to occlude the vessels found in the esophageal wall. At this stage in the operation, since its blood supply has been completely cut off, the section of the gastrointestinal tract intervening between the cannulae is removed along with the spleen and pancreas by severing the mesenteric attachments to the dorsal wall of the abdominal cavity and to the liver.

The next step is the removal of the liver. The mesentery attaching the liver to the dorsal abdominal wall is divided by blunt dissection and then a ligature is tied over the original ligature on the vena cava in such a way as to include the small adhesions that form after the preliminary operation and which tend to bleed slightly if not tied off. The falciform and triangular ligaments and the portion of the lesser omentum between the esophagus and liver are divided and a ligature is placed on the vena cava just cranial to the point at which it leaves the liver. The liver along with the portion of vena cava intervening between these ligatures is then removed. A button of liver weighing about 50 mgm. must be left on the cranial stump of the vena cava to prevent the movements of the diaphragm from slipping the ligature off the stump of the vena cava. It is extremely unlikely that this bit of liver exerts any influence on the fate of the preparation for not only is it small but also it is separated from the circulating blood by a ligature.

If the kidneys are to be removed, a noose of silk is slipped over each and tied

tightly around the vessels. The kidneys can then be dissected away with a minimal loss of blood. Care is taken to leave the adrenal glands as intact as possible. If on the other hand the adrenals are to be removed, they are similarly tied off and removed along with their enveloping adipose tissue.

The operative procedures are completed by closing the abdominal wall in a single layer. Size "C" serum proof silk is a convenient size and type of material to use for ligatures and suture material throughout. With practice the operation should be performed in 15 to 25 minutes.

Analytical procedures. The blood sugar determinations unless otherwise specified were carried out by a micro ferricyanide method using the filtrate from 0.02 cc. of blood from which the proteins were precipitated by tungstic acid (Reinecke, 1942b). When micro fermentation is to be used in conjunction with this method to determine the fermentable reducing substance in blood, 0.05 cc. of blood is measured into the 5 cc. of the dilute tungstic acid reagent rather than the 0.02 cc. used otherwise. Two cubic centimeters of the filtrate obtained is diluted with 3 cc. of distilled water. The blood sugar determination is then carried out on this diluted, unfermented filtrate in the usual way. The conventional blood sugar value thus obtained represents the sum of the fermentable and non-fermentable reducing substances in the blood that are not removed by the treatment with the tungstic acid reagent.

The remainder of the undiluted filtrate from the 0.05 cc. sample of blood is fermented with baker's yeast to give the glucose equivalent of the non-fermentable reducing substance. Fleischmann's yeast of the type sold in 1 lb. bricks is washed thoroughly with water. One part of yeast is suspended in four parts of water by vigorous stirring. The yeast is then centrifuged down and the water decanted. This is repeated ten times. The yeast is then made up in a 20 per cent suspension with distilled water and is used within an hour or two. The suspension is measured in 0.5 cc. portions into 11 x 60 mm. culture tubes which are then centrifuged at high speed. The supernatant water is removed with a fine glass capillary attached to a suction pump. The remaining filtrate, which amounts to about 2.5 cc., is poured onto the yeast. The yeast is resuspended by vigorous shaking. A square of "Parafilm" held in place with the finger tip may be used to close the open end of the tube. The yeast is then incubated with the filtrate for about 45 minutes in a water-bath held at 30°C. The tubes are shaken to resuspend the yeast about twice during this period. Then the yeast is again centrifuged down at high speed. Two cubic centimeters of the supernatant fluid are carefully removed with a pipet and diluted with 3 cc. of distilled water. The glucose equivalent of the non-fermentable reducing substance is then determined in this diluted, fermented filtrate. This value is corrected for the amount of reducing substance contributed by the yeast itself by subtracting the value obtained from a blank made up in a comparable manner from distilled water that had been incubated with a portion of the yeast concurrently with the filtrate. This blank usually gives a value equivalent to less than 10 mgm. per cent of glucose in the original blood. The difference between this corrected glucose equivalent of the non-fermentable

reducing substance and the conventional blood sugar value is taken as the glucose equivalent of the fermentable reducing substance in the blood. Under the circumstances of the experiment it is probable that this was largely glucose. As an additional check on the procedure, it has been our custom to ferment solutions containing glucose in excess of that to be found in the filtrates, concurrently with the filtrates. No significant amounts of sugar have remained in these solutions.

RESULTS. *The influence of blood loss and the administration of fluid on the blood sugar levels and survival of the non-nephrectomized, eviscerated rat.* In as

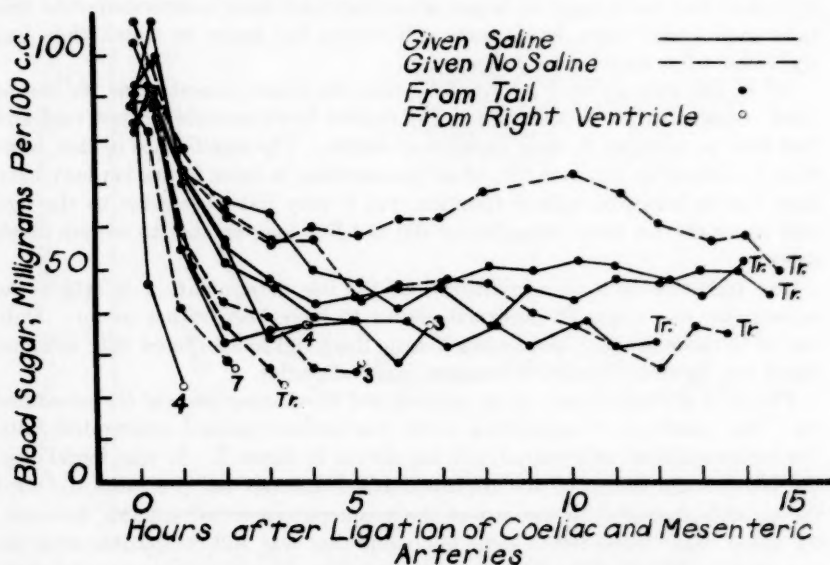


Fig. 1. *The influence of blood loss and the administration of fluid on the blood sugar levels and survival of the non-nephrectomized, eviscerated rat.* The animals given physiological saline received 2 cc. subcutaneously and 1 cc. intravenously (via the venous spaces of the penis) per 100 grams of body weight. The solution was given immediately after the completion of the operative procedures. The number shown near the final plotted value for each animal represents the approximate blood loss in grams. Tr. indicates that only traces of blood were lost. All the animals were eviscerated within a period of five hours.

much as Bergman and Drury had found that only the kidneys that were actively secreting urine were effective in reducing the apparent glucose consumption of the eviscerated rabbit, this experiment was planned to determine if the administration of physiological salt solution immediately after the evisceration would act as a diuretic and perchance improve the viability of the preparation. Quite accidentally during and after the eviscerations, several of the animals suffered an appreciable loss of blood. This was estimated by weighing the bits of cotton used to sponge up the shed blood. The results as presented in figure 1 indicate that loss of a few cubic centimeters of blood markedly reduces the survival of

this preparation and that, surprisingly enough, it seems that the cause of death is hypoglycemia. This is suggested not only by the low sugar levels found at death but also by the fact that all of the animals dying before seven hours after evisceration exhibited convulsions. This type of convulsion in the previous experience of the author has always been relieved by the injection of glucose.

The concentration of sugar in the blood at first decreased and then remained nearly constant in those animals that survived for ten or more hours. Unfortunately it was impossible to observe all of the animals continuously until they died, but two were alive 21 hours after they had been eviscerated. At this time their blood sugar levels were still within the range at which they had stabilized a few hours after evisceration.

All of the animals that survived beyond ten hours passed urine on one or more occasions after evisceration. The shorter lived animals did not void and had little or no urine in their bladders at death. The significance of this, however, is difficult to evaluate for, while this decrease in urine formation may have been due to impaired kidney function, yet it may have been due to the fact that these shorter lived animals just did not live long enough to secrete much urine.

The influence of fluid administration, for the determination of which the experiment was originally designed, seems to have been rather minor. Only one of the animals that had been given no fluid and had suffered only minimal blood loss showed the expected shorter survival period.

The effect of nephrectomy on the survival and blood sugar levels of the eviscerated rat. The results of a comparison of the non-nephrectomized eviscerated, with the nephrectomized eviscerated, rat are shown in figure 2. It was found that the blood sugar levels of the nephrectomized animals fell precipitously until the animals expired. In the case of the non-nephrectomized animals, however, the blood sugar levels fell to some low value that was still compatible with life and then stabilized there for many hours before the animals eventually died. The apparent blood sugar levels of the non-nephrectomized animals were higher at death than those of the nephrectomized animals. It must be recalled, however, that the method used for determining the blood sugar includes some non-fermentable reducing substances. The fact that animals in both groups exhibited convulsions before death strongly suggests that they died of hypoglycemia in both cases.

The rôle of the adrenal in maintaining the blood sugar in the eviscerated rat. Because nephrectomy is very likely to interfere with the normal function of the adrenal gland and because of the known importance of this gland in carbohydrate metabolism, adrenalectomized, eviscerated rats were compared with similar non-adrenalectomized animals. The results shown in figure 3A seem to indicate that the adrenal is not of major, immediate importance in maintaining the blood sugar concentration in the blood of the eviscerated rat. It is true that the adrenalectomized animals showed a greater decrease in blood sugar than did comparable non-adrenalectomized animals, but while it is possible that this

may be a specific effect, yet it is also likely that it may be incidental to some impairment of renal circulation due to the manipulations performed in adrenalectomy. The material presented in figure 3B is taken from the unpublished data of Reinecke and Kendall. It shows that the injection of adrenal cortical extract is without major acute effect on the blood sugar of the eviscerated rat. This result was also obtained by Selye and Dosne (1940), but the characteristics of the preparation used by these investigators were so different that it is doubtful if their data are comparable.

The effect of the kidney on the concentration of sugar in the blood flowing through it. The influence of a functioning kidney on the concentration of sugar in the

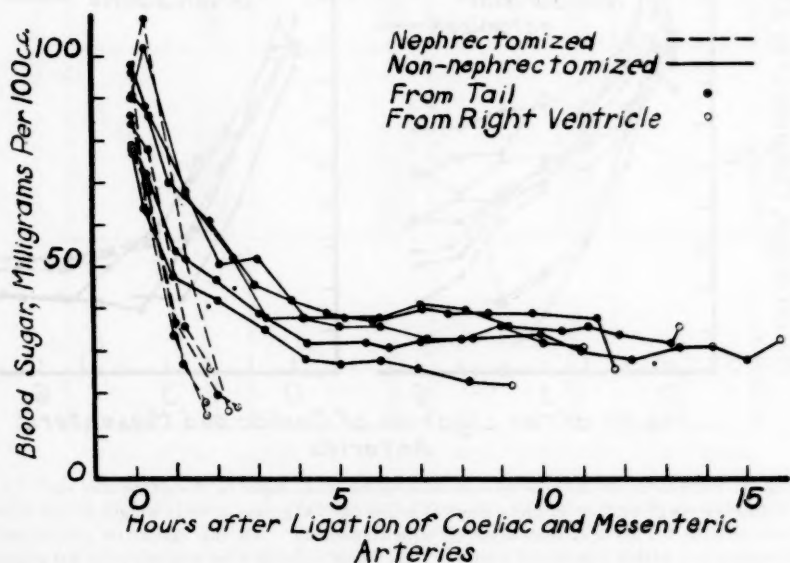


Fig. 2. The effect of nephrectomy on the survival and blood sugar level of the eviscerated rat. All animals were given 2 cc. of physiological saline per 100 grams of body weight at the time of evisceration. This was administered subcutaneously. All the eviscerations were completed within 7 hours.

blood of the eviscerated rat might possibly be explained in three ways: 1, the kidney itself may act as a source of blood sugar; 2, the presence of the kidney may allow other tissues to act as a source of blood sugar; 3, the presence of the kidney may lead to the utilization of glucose at a slower rate than occurs in its absence. The application of Occam's "Razor" suggests the first possibility. (Bergman and Drury, however, postulated the third alternative because they found that tying off the ureters without interfering with the blood vessels of the kidneys led to an apparent increase in the rate at which glucose was utilized. From this they argued that the kidneys were excreting some substance which stimulated glucose utilization.) A study of the sugar concentrations in the blood

of the aorta and renal vein of the eviscerated rat was therefore undertaken to determine whether or not the first possibility might afford an explanation. The results are shown in figure 4. The concentration of sugar-like reducing substance was consistently found to be higher in the renal vein blood and lower in the vena caval blood (with one exception that may be experimental error) than in the aortic blood. That these differences are due to actual differences in the amount

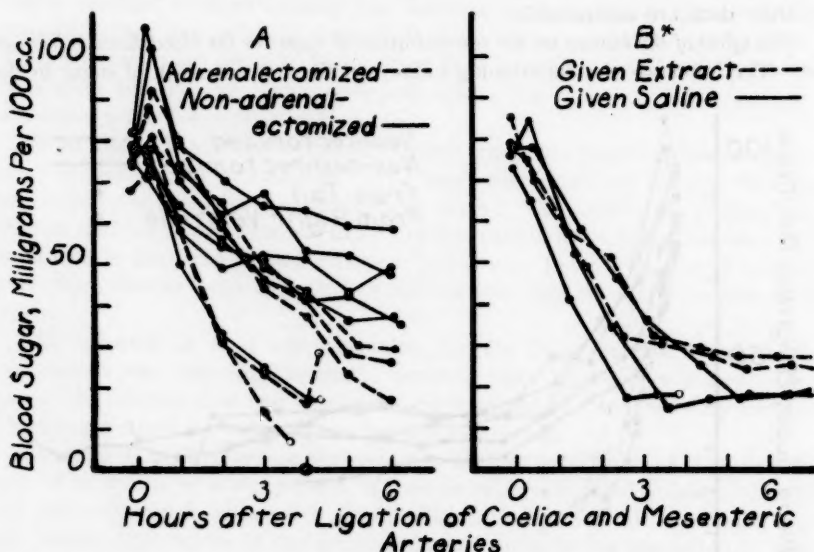


Fig. 3. The role of the adrenal in maintaining the blood sugar in the eviscerated rat. (A) All animals were given 2 cc. of physiological saline per 100 grams of body weight at the time of evisceration. This was administered subcutaneously. All the operative procedures were completed within a period of 5 hours. (B) These animals were slightly over 200 grams in weight at the time of evisceration. All were given 1 cc. of physiological saline per 100 grams of body weight and a dose of phenolsulfonphthalein intravenously shortly after evisceration; 5 cc. of an aqueous solution containing the extract of 75 grams of adrenal gland per cc. was administered subcutaneously immediately after the intravenous injection to the indicated animals. The others were given a corresponding amount of physiological saline in a similar fashion. The methods used were comparable in general to those described in this paper.

* These data are taken from some unpublished studies of R. M. Reinecke and E. C. Kendall of the Mayo Clinic, Rochester, Minnesota.

of glucose present, at least with respect to the renal vein and aortic blood, is strongly suggested by the fact that they were also apparent when the fermentable reducing substance was determined.

An incidental finding was that the concentration of reducing substance in the renal vein blood tends to increase in consecutive samples taken at short intervals.

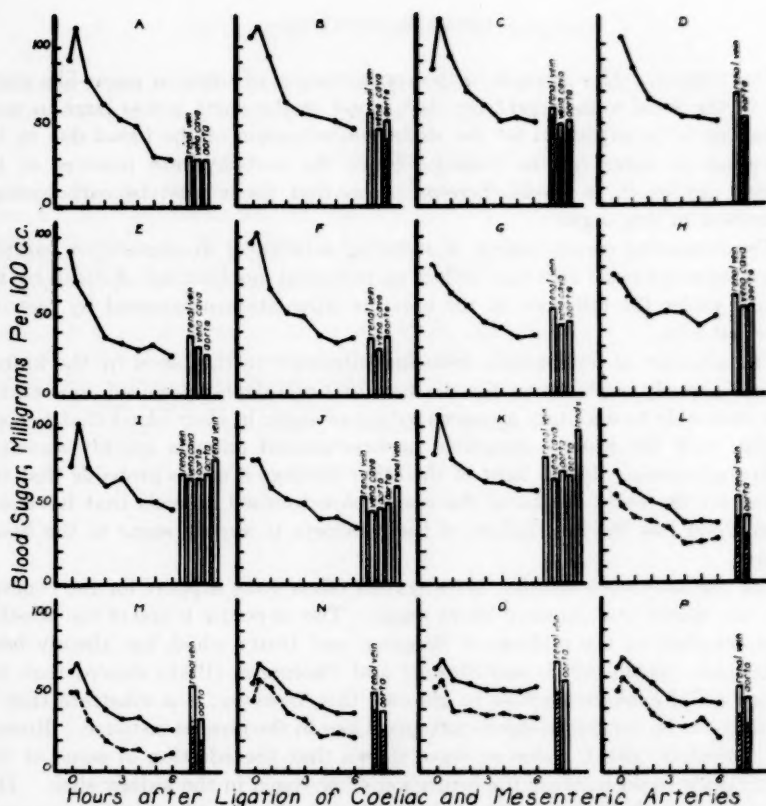


Fig. 4. The effect of the kidney on the concentration of sugar in the blood flowing through it. All animals were given 2 cc. of physiological saline per 100 grams of body weight subcutaneously at the time of evisceration. The arterial and venous samples were taken with the animals under amytal anesthesia (9 mgm. per 100 grams body weight were given subcutaneously). (A-F) Large samples, 0.5-1.0 cc., were drawn simultaneously from the vessels indicated. (G-K) Small samples were obtained by puncturing the indicated vessel with a no. 27 hypodermic needle and collecting the blood in a capillary pipet from the small pool that formed. Samples were taken consecutively in the order shown by the bars from left to right. (L-P) Small samples, 0.10-0.15 cc. were drawn simultaneously with 0.25 cc. hypodermic syringes.

The heights of the entire bars represent the glucose equivalent of the reducing substances in the tungstic acid filtrate of the blood samples as determined by the micro blood sugar method referred to. Solidly shaded portions correspond to the values obtained with the methods described by Somogyi (1930, 1937). The cross-hatched portions represent fermentable reducing substance determined in the manner described in this article. The solid-line curves represent glucose equivalents obtained in the same way as those represented by the heights of the entire bars. The broken-line curves like the cross-hatched portions of the bars represent fermentable reducing substance. The samples represented by the bars were taken within a few minutes after the last samples represented in the curves. The lack of shading or cross-hatching in the majority of the bars indicates that the corresponding determinations were not made in these instances.

The arteriovenous differences of the glucose equivalents of the fermentable reducing substance for L, M, N, and P were respectively 11, 9, 13, and 6 mgm. per cent.

The three groups, A-F G-K, and L-P were studied separately on three different days.

DISCUSSION. The increase in the concentration of sugar or sugar-like material in the renal vein blood over that found in the aorta is too large in most instances to be explained by the slight concentration of the blood due to the excretion of water by the kidney. Since the carbohydrate reserves of the kidney are small, it would therefore seem that there must be carbohydrate formation in this organ.

The increasing concentration of reducing substances in consecutive samples taken from the renal vein may reflect an increased mobilization of sugar by the kidney under the influence of the nervous stimulation occasioned by piercing the renal vein.

The addition of fermentable reducing substance to the blood by the kidney affords a ready explanation for the fact that non-nephrectomized, eviscerated rats were able to maintain a concentration of sugar in their blood that is compatible with life while comparable nephrectomized animals quickly succumb to hypoglycemia. In the light of the other findings it seems probable that the reason for the early deaths of the non-nephrectomized animals that have suffered blood loss lies in a failure of their kidneys to supply sugar to the blood stream.

The previous work of other investigators offers some support for the concept that the kidney is a source of blood sugar. This of course is one of the possible interpretations of the findings of Bergman and Drury which has already been mentioned. Goda (1938) and Stewart and Thompson (1941) showed that the kidney could convert fructose to glucose; this, however, is a substrate that is not likely to be present in significant quantities in the present instance. Russell and Wilhelmi (1941), however, have shown that the addition of some of the amino acids could increase the formation of glycogen in the kidney slice. The amino acids probably are available in the preparation used in these experiments and are one of the probable sources from which the kidney forms glucose.

It is of interest to note that Himsworth (1938) and Himsworth and McNair Scott (1938) obtained some evidence that there are extra-hepatic sources of blood sugar in the hepatectomized rabbit. The results of the experiments just described would of course suggest that the source of blood sugar may have been the kidneys.

SUMMARY

In the presence of the kidneys the concentration of sugar in the blood of the eviscerated rat at first falls but then remains at a nearly constant value for a period of many hours.

Loss of blood causes the prompt development of hypoglycemia in the non-nephrectomized, eviscerated rat.

The concentration of sugar in the blood of the nephrectomized, eviscerated rat falls precipitously until the animal dies.

Adrenalectomy or the administration of adrenal cortical hormones is without major, acute effect in the non-nephrectomized, eviscerated rat.

During the period in which the eviscerated rat is able to maintain the con-

centration of sugar in its blood at a nearly constant value, fermentable reducing substance is added to the blood as it flows through the kidneys.

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